O·I·Analytical

Application Note 25570306

Fipronil Analysis By GC/XSD following Post-Extraction Gel Permeation Chromatography Cleanup

Abstract

Fipronil is an emerging broad-spectrum insecticide often used as an alternative to chloropyrifos. Fipronil will degrade, under certain conditions, to compounds that can be more toxic to non-selected species than the parent compound. Gel permeation chromatography (GPC) is a common post-extraction cleanup procedure for removing high molecular weight interferences prior to pesticide analysis, but there is little published information on its effectiveness for fipronil. The purpose of this study was to evaluate the effectiveness of a standard GPC cleanup protocol prior to the analysis of fipronil using a halogen-specific GC detector. GPC cleanup was achieved using an OI Analytical Model AP2000 GPC Cleanup System (Figure 1). Extracts were evaporated to dryness before and after GPC cleanup using a Labconco RapidVap System. Analysis was by GC with an OI Analytical Model 5360A Halogen Specific Detector (XSD).

Keywords

Gel Permeation Chromatography
GPC AutoPrep 2000
Pesticides
RapidVap N2 System
XSD
GPC
Fipronil

Figure 1. GPC AutoPrep 2000 Gel Permeation Chromatography System

REGISTERED COMPANY ISO 9001 SGS

Introduction

Fipronil is a member of a new class of pesticides known as phenylpyrazoles. It is used against lepidopterous and orthopterous pests on a wide variety of crops and against pests on golf courses and commercial turf. Fipronil also is used for termite control under the market name Termidor® and for the control of fleas, ticks and mites on domestic animals under the market names Frontline® or Frontline Top Spot®. Fipronil and its degradation products (Figure 2) are highly toxic to fish, aquatic invertebrates, and upland game birds. It is moderately toxic to small mammals if ingested. Fipronil sulfone, which is formed through aerobic soil metabolism, is more toxic than fipronil to birds, freshwater fish and invertebrates.

Published methods for the analysis of fipronil from food products typically utilize a liquid/liquid extraction, a cleanup step with C-18 solid phase extraction (SPE) or column chromatography, and quantitation using gas chromatography/mass spectrometry (GC/MS) or HPLC with UV diode array detection. Gel permeation chromatography (GPC) has been extensively used as an effective post-extraction cleanup procedure for removing high-molecular-weight interferences from complex sample matrices such as food products prior to pesticide analysis. It is especially effective at removing lipids, pigments and proteins compared to SPE. There is little published information on the effectiveness of GPC cleanup for the analysis of fipronil and its primary degradation products. The purpose of this study was to determine the feasibility of utilizing a standard GPC cleanup protocol prior to fipronil analysis by GC with a halogen specific detector (GC/XSD) rather than the standard cleanup procedures and GC/MS analysis.

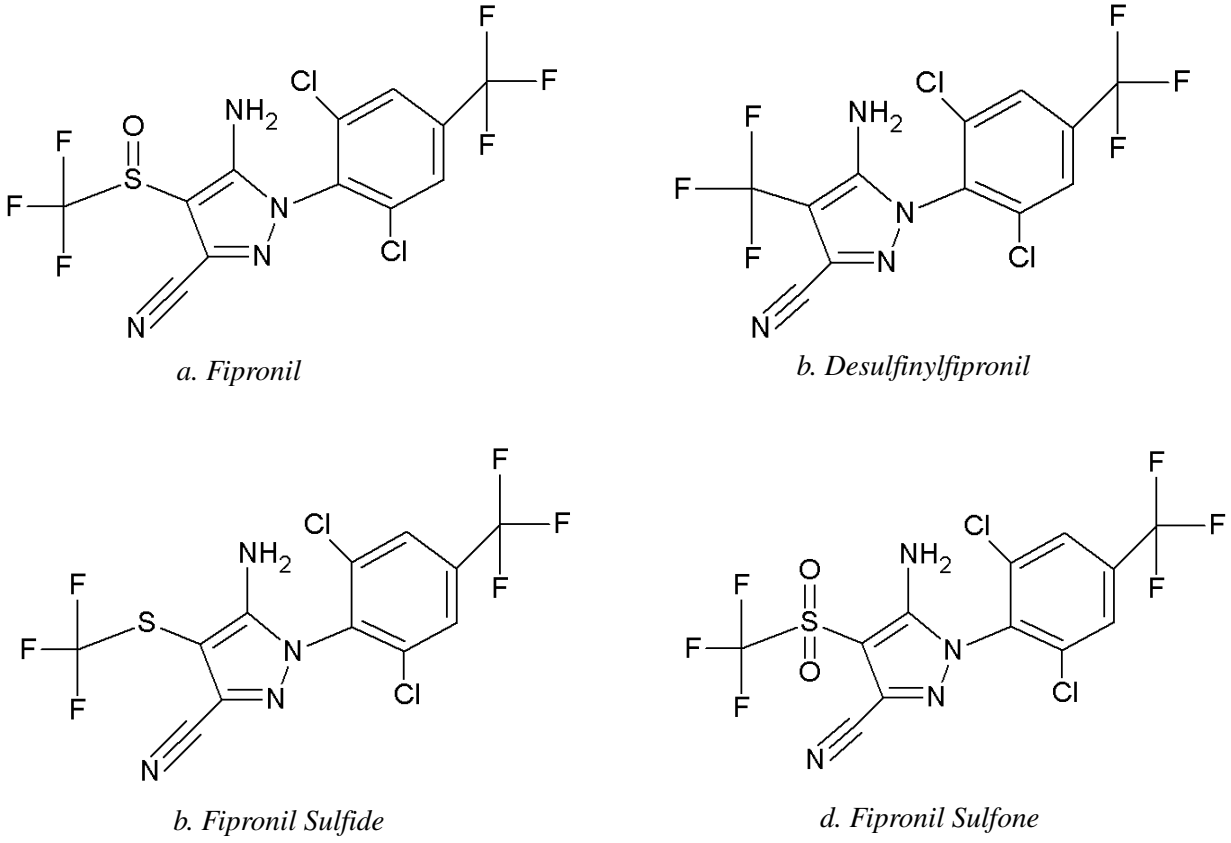


Figure 2. Fipronil and its Breakdown Products

Experimental

Materials

All solvents were distilled in glass suitable for HPLC, GC, pesticide residue analysis and spectrophotometry. All chemicals were ACS grade or better. Fipronil, fipronil sulfone, fipronil sulfide and desulfinyl fipronil were obtained from AccuStandard, Inc. (New Haven, CT). GPC standards were prepared according to USEPA Method 3640A and contained corn oil, bis(2-ethylhexyl) phthalate, methoxychlor, perylene and sulfur.

Sample Preparation

Lettuce samples were homogenized and an aliquot of 20 grams was placed in a blender. The lettuce was then fortified with varying concentrations of fipronil, or no fipronil for the blank. Eighty mL of ethyl acetate and 50 grams of sodium sulfate were added, and the samples were blended for two to three minutes. The extract was filtered through Whatman No. 40 paper and then concentrated to < 1 mL using a Labconco N2 Rapidvap system (Figure 3). The residue was then reconstituted in 10 mL of GPC mobile phase (1:1 ethyl acetate:cyclohexane) prior to GPC cleanup.

Figure 3. RapidVap N2 Evaporation System

GPC Cleanup

GPC cleanup was achieved using the GPC AutoPrep 2000 (Figure 1) equipped with a 700 mm X 25 mm glass column containing 60 grams of Envirobeads™ S-X3 resin. The system used a 5 mL sample loop and a flow rate of 5 mL/minute, with a 50:50 mixture of ethyl acetate:cyclohexane as the mobile phase. The GPC column was calibrated by using the calibration standard described above, an OI Analytical UV Detector set at 254 nm, and WinSEP software (Figure 4). Based on the UV trace, column eluate collection began just before bis (2-ethylhexyl)phthalate elution and after corn oil elution (Figure 5). Eluate collection stopped after perylene elution. After GPC cleanup the collected fractions were evaporated to 1.5 mL using a Labconco Rapidvap N2 system and injected into the GC for analysis.

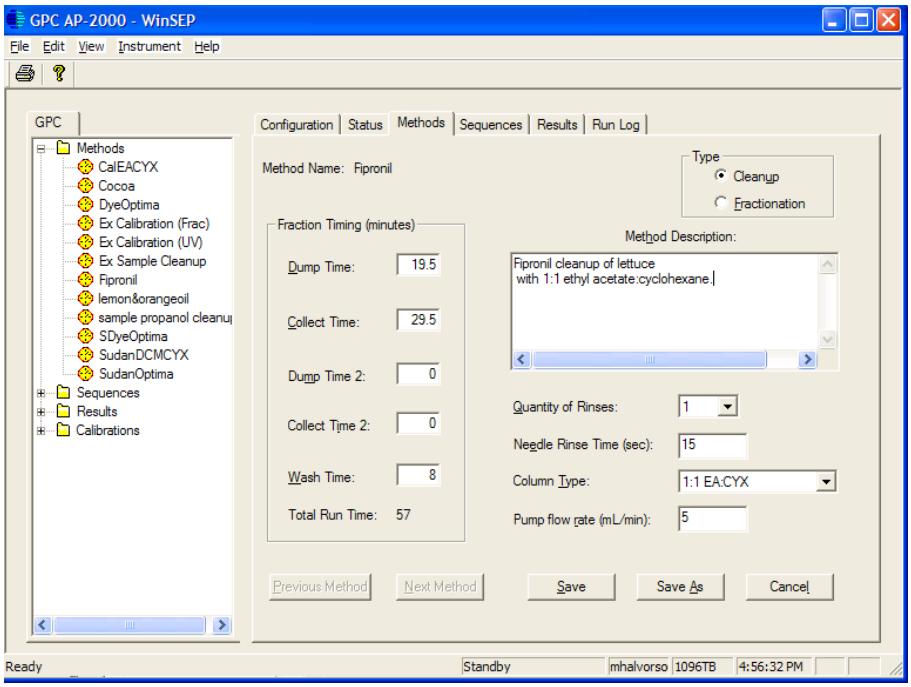


Figure 4. Screen from the WinSEP Software User Interface Illustrating Programming Steps for the Fipronil Cleanup

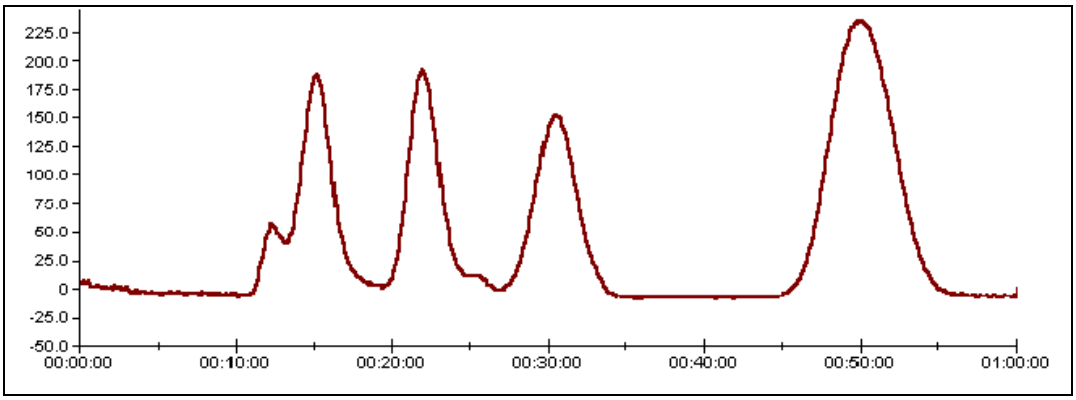
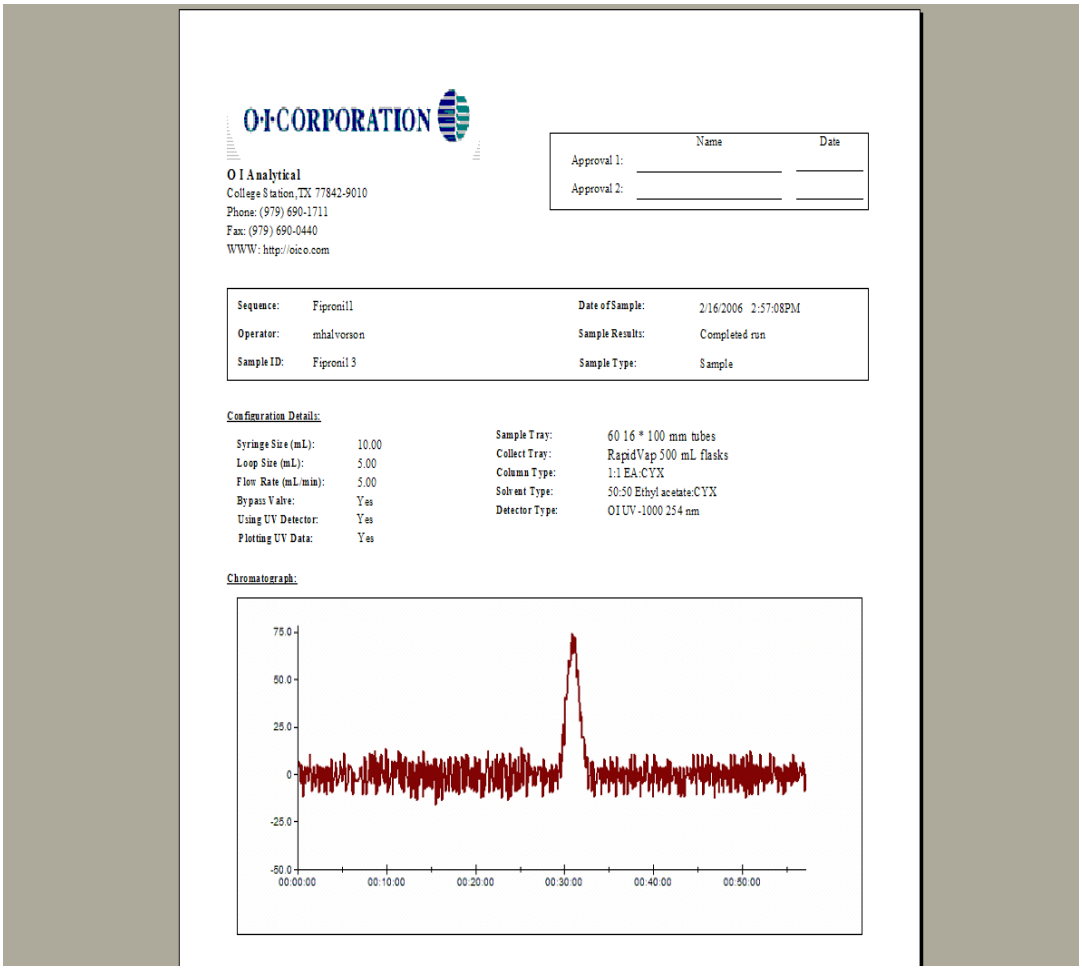


Figure 5. GPC chromatogram of the USEPA Method 3640A calibration standard using 1:1 cyclohexane/ethyl acetate and an OI Analytical UV Detector (254 mm, 1.000AUFS)

GC Analysis

Fipronil and its degradation products were analyzed using an Agilent Series 6890 GC with a Agilent DB-5MS column and an OI Analytical Model 5360A Halogen Specific Detector (XSD™).

RESULTS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	Name	Date
Approval 1:		
Approval 2:		

Sequence:	Fipronil1	Date of Sample:	2/16/2006 2:57:08PM
Operator:	mhalvorson	Sample Results:	Completed run
Sample ID:	Fipronil 3	Sample Type:	Sample

Configuration Details:

Syringe Size (mL):	10.00	Sample Tray:	60 16 * 100 mm tubes
Loop Size (mL):	5.00	Collect Tray:	RapidVap 500 mL flasks
Flow Rate (mL/min):	5.00	Column Type:	1:1 EA:CYX
Bypass Valve:	Yes	Solvent Type:	50:50 Ethyl acetate:CYX
Using UV Detector:	Yes	Detector Type:	OI UV-1000 254 nm
Plotting UV Data:	Yes		

Chromatograph:

Figure 6. Chromatogram of a lettuce extract fortified with ~3 µg/mL on the AutoPrep 2000 GPC cleanup system using an Envirobeads S-X3 column with a 50:50 ethyl acetate:cyclohexane mobile phase at 5 mL/min, WinSEP software and the OI Analytical UV detector (254nm, 1.000 AUFS). Fipronil elutes at approximately 30 minutes

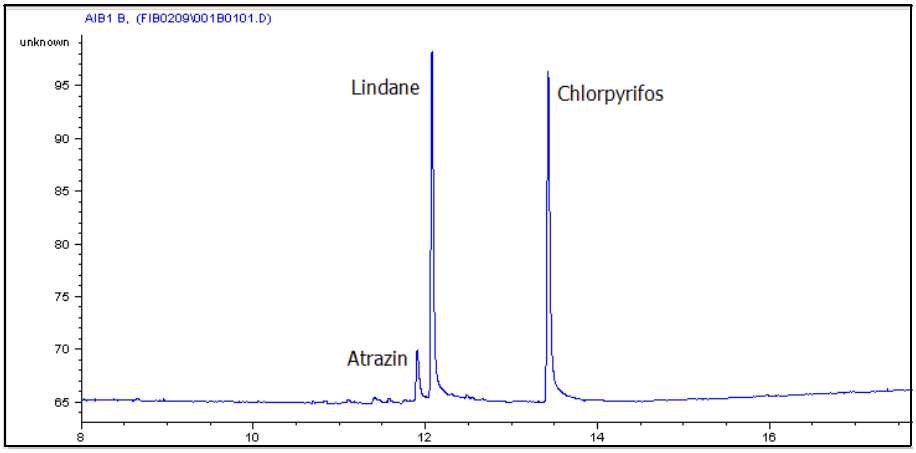

Figure 7. GC/XSD chromatogram of a mixed standard containing azobenzene, thimet (or phorate), atrazine, lindane, diazinon and chlorpyrifos after GPC cleanup. The XSD only detects compounds containing halogens such as chlorine or fluorine

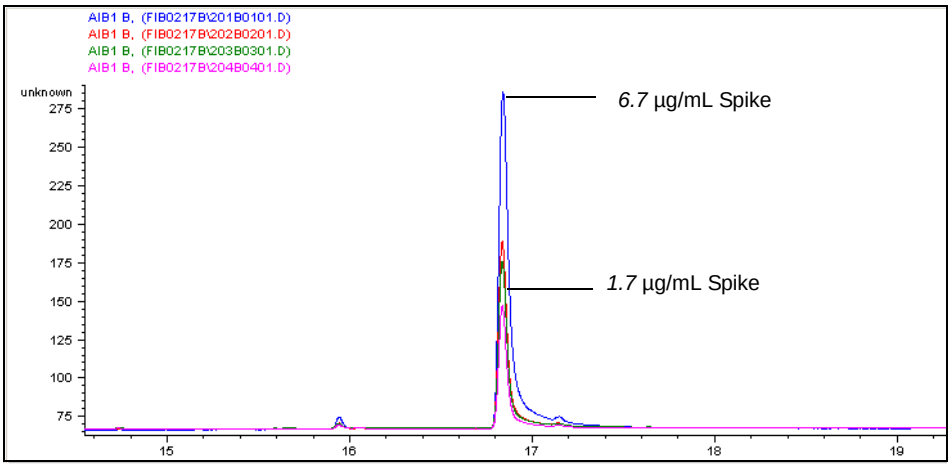

Figure 8. GC/XSD chromatogram of Fipronil in fortified lettuce after extraction, GPC cleanup and concentration. Fipronil was spiked at four different levels from 1.7 µg/mL to 6.7 µg/mL

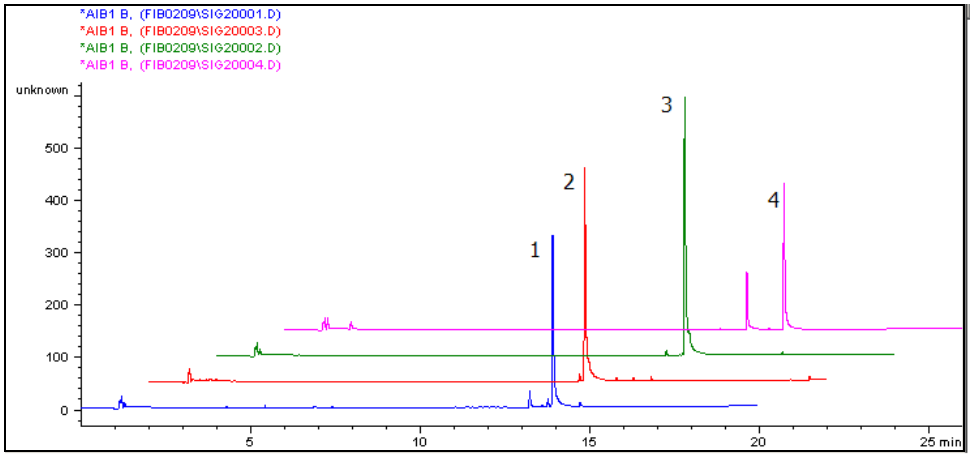

Figure 9. Individual chromatograms of desulfinyl fipronil (1), fipronil sulfide (2), fipronil (3), and fipronil sulfone (4) using an Agilent DB-5MS column (30 meters x 0.25 mm I.D. x 1.0 µm film thickness) with oven program 50 ºC (hold for 1 min), 15 ºC/ minute to 260 ºC (hold for 5 minutes)

Table 1. Individual Retention Times of Fipronil Standards on GC/XSD System

Elution Order	Compound	Retention Time (min.)
1	Fipronil desulfinyl	12.95
2	Fipronil sulfide	13.79
3	Fipronil	13.94
4	Fipronil sulfone	14.79

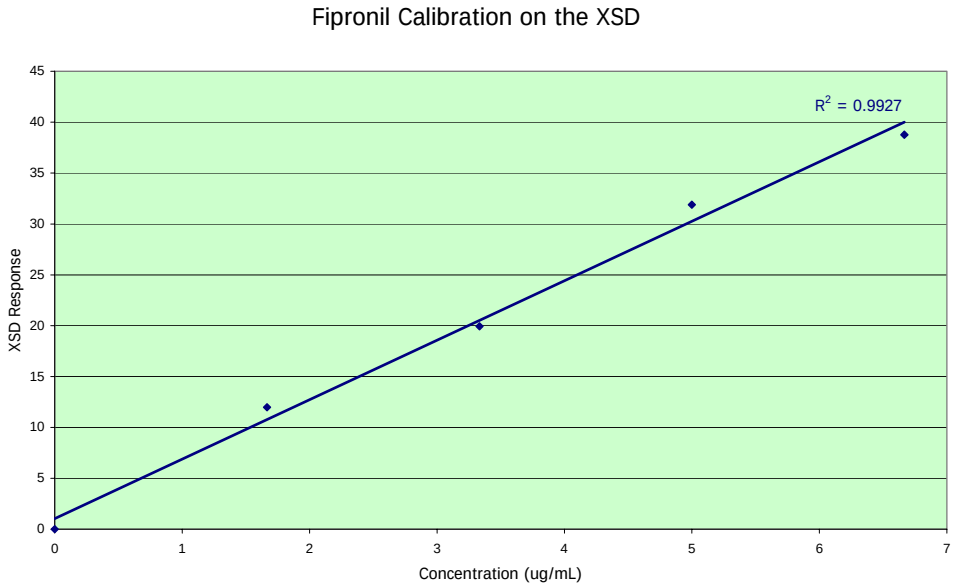

Figure 10. Regression Analysis of fipronil fortified at increasing concentrations in lettuce followed by extraction, GPC cleanup, concentration, and analysis by GC/XSD.

SUMMARY AND CONCLUSIONS

The GPC AutoPrep 2000 was a highly effective and efficient tool for the cleanup of food products such as lettuce prior to Fipronil analysis. Fipronil eluted from the GPC column at approximately 30 minutes using a column packed with 60 grams of Envirobeads S-X3 and a mobile phase containing 1:1 ethyl acetate:cyclohexane at a flow rate of 5 mL/min. It was not necessary to use chlorinated solvents for either the extraction steps or the GPC cleanup thus preventing exposure to chlorinated solvents and the costs to properly dispose of chlorinated solvent waste. The XSD detector provided sensitive and specific detection of fipronil and its degradation products due to the presence of both fluorine and chlorine. Studies are currently underway to evaluate GPC cleanup of other foods and animal tissue prior to analysis for fipronil and its degradation products

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