Analysis of the Active Compound in an Agricultural Fungicide Formulation by Liquid Chromatography

Application

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Abstract

Liquid chromatography was shown to be an excellent tool for the routine analysis of the active ingredient in an agricultural fungicide formulation. No extensive sample preparation or cleanup was required to remove the active ingredient from the rest of the sample matrix. The active ingredient was easily separated and detected using conventional reverse-phase conditions with a UV/VIS detector.

Introduction

Agricultural chemical formulations usually contain an active ingredient and several inert components, such as surfactants, that are designed to enhance the efficacy of the product. Gas chromatographic analysis of these formulations cannot be performed due to the polarity or thermal instability of the active ingredient as well as the high molecular weight and polarity of the surfactants. Therefore, liquid chromatography offers the best solution for the routine analysis of the active ingredients in an agricultural formulation.

This work was done on a commercially available fungicide formulation. The active ingredient in this product is 6.5 % (wt) of N,N-[1,4-piperazinediylbis (2,2,2-trichloroethylidene)] bisformamide. This is also known as triforine (CAS registry number 26644-46-2) and the structure is shown in Figure 1. The "inactive" ingredients in this formulation are listed as cyclohexanone, N-methyl pyrrolidone and Atlox 3406-F. The Atlox 3406-F is an agricultural dispersant that contains ionic and nonionic surfactants and mixed aromatic solvents. Electrospray ionization liquid chromatography/mass spectrometry (LC/MS) analysis has shown that the triforine can be easily separated and identified in the formulation [1].

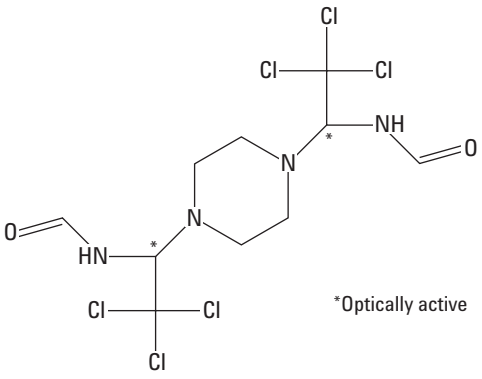

Figure 1. Chemical structure of triforine, the active ingredient in some commercial fungicide formulations.

Experimental

A 10% (v/v) solution of the fungicide formulation was made in acetonitrile. This solution was run on the Agilent 1100 Series LC System. This system included a vacuum degasser, a binary pump, an autoinjector, a thermostated column compartment, and a diode array UV/VIS detector. LC instrument conditions for this analysis are shown in Table 1.

Table 1. LC Analysis Conditions

Liquid chromatograph conditions	
Column:	Zorbax® XDB-C8,150 × 4.6 mm, 5 µm (p/n 993967-906)
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	Acetonitrile
Mobile phase gradient:	30% B at 0 min, 50% B at 7 min, 95% B at 10 min
Flow rate:	1.0 mL/min
Injection volume:	1 mL
Column temperature:	30 °C
Detector:	Diode array
Signal wavelength:	254 nm
Signal bandwidth:	10 nm
Reference wavelength:	500 nm
Reference bandwidth:	40 nm

Results and Discussion

Figure 2 shows the chromatogram of the fungicide formulation. The active ingredient in the formulation, triforine, elutes as two chromatographic peaks between 7.5 minutes and 7.8 minutes. The presence of two triforine peaks is due to the stereochemistry of the structure. Figure 3 shows the four triforine stereoisomers. These four configurations can be grouped into two pairs of mirror images that are diastereoisomers. The S,R and R,S configurations are mirror images that are superimposable, resulting in a meso compound that exhibits no optical activity or differences in physical properties. Therefore, because the S,R and R,S configurations are identical, they will elute as one chromatographic peak. The second pair of mirror images are the R,R and S,S configurations. These are not superimposable and are, therefore, enatiomers that will have different optical activity, but identical physical properties. Conventional reverse-phase liquid chromatography cannot separate these enantiomers, and they will co-elute as a single peak. However, these enantiomers can be separated from the meso compound by reverse-phase LC. This is why there are two triforine peaks, one for the meso compound and one for the enatiomers. Without pure standards of the stereoisomers, it is not possible to determine which configurations can be attributed to the observed chromatographic peaks.

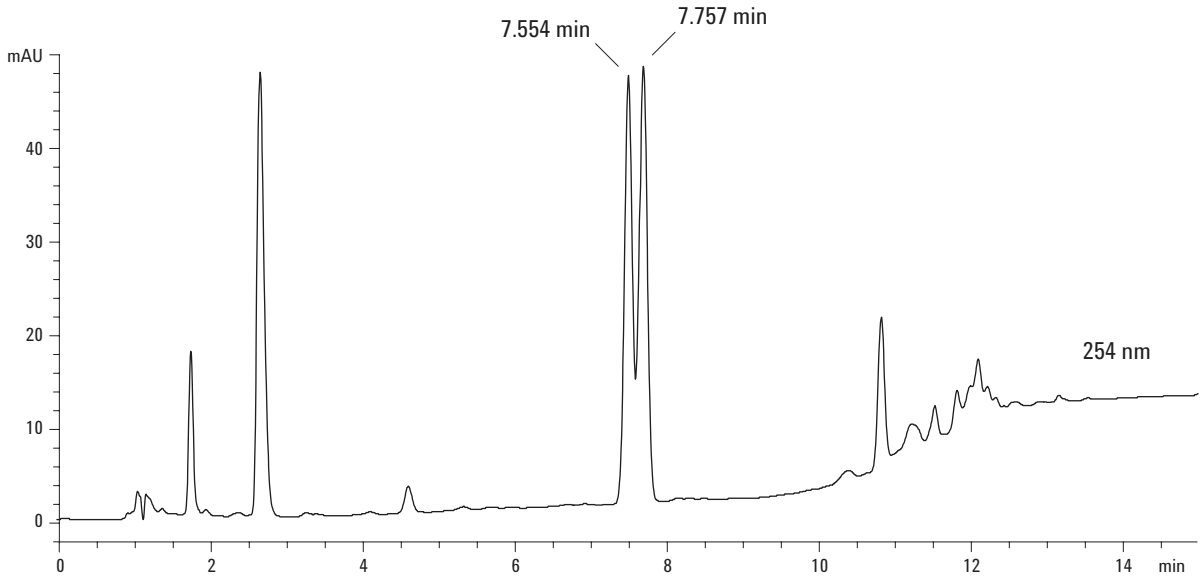

Figure 2. LC of an agricultural fungicide formulation containing the active ingredient triforine. The two peaks at 7.554 min and 7.757 min were shown to be optical isomers of triforine.

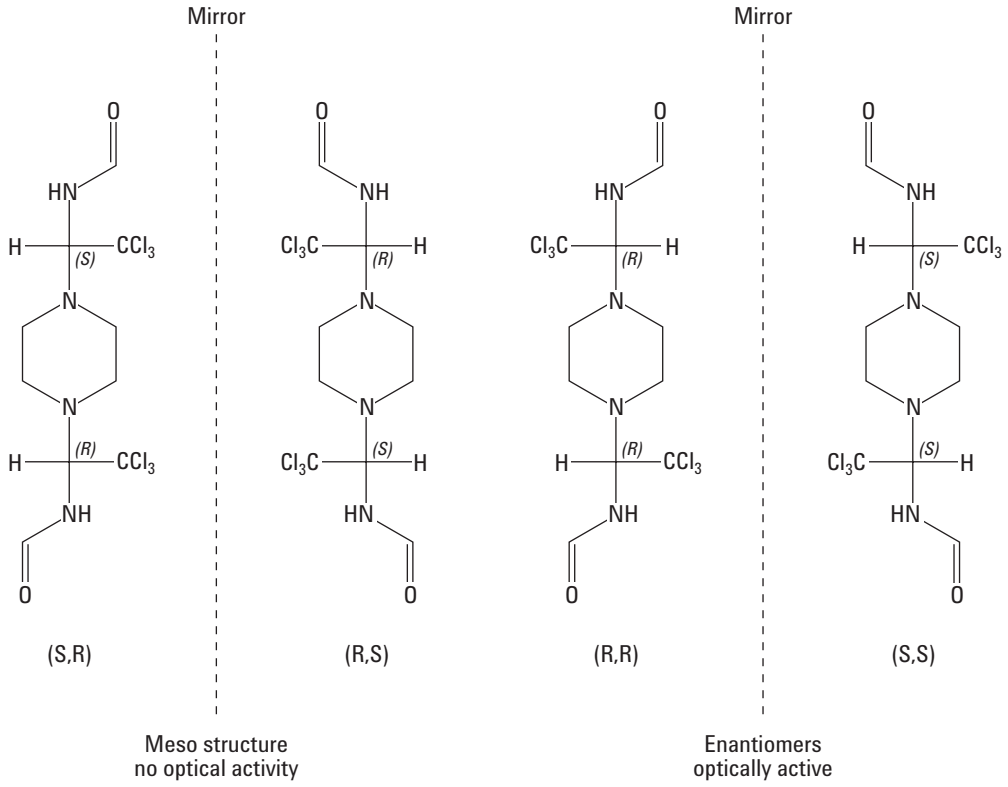

Figure 3. The four triforine stereoisomers arising from the two chiral carbons in the structure. These two pairs of mirror images account for the two triforine peaks observed in the chromatogram.

Conclusions

Liquid chromatography was shown to be an excellent tool for the routine analysis of the active ingredient in an agricultural fungicide formulation. No extensive sample preparation or cleanup was required to remove the active ingredient from the rest of the sample matrix. The active ingredient was easily separated and detected using conventional reverse-phase conditions with a UV/VIS detector.

References

1. McCurry, J.D., and Zavitsanos, P., "Analysis of Components, Contaminants, and Impurities in Fungicide Chemical Formulations by GC/MS and LC/MS," Agilent Technologies Application Note, Publication 5988-6085EN, April 2002.

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