Waters Alliance® LC/MS System

LC/MS Analysis of Common Fungicide Residues

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Key Words
Fungicides
Benomyl
Carbendazim
Thiabendazole
XTerra™ column
Electrospray
Resides
Sample clean up

Introduction

Systemic fungicides such as thiabendazole and carbenazim, a degradation product of benomyl, are used to control mold growth on fruits and vegetables. Sensitive analytical methods are required to determine the presence or absence of resides of these compounds. HPLC separations with UV or photodiode array detection (PDA) can be difficult because of the low levels of the residues and the complexity of the sample matrix, e.g. fruit juice. By adding a single quadrupole mass spectrometer as a detector, high sensitivity detection, quantitation can be achieved in one analytical run.

Analytical Conditions

Instrumentation:	Waters Alliance® LC/MS system consisting of a 2690 Separations Module, 996 photodiode array detector and ZMD mass detector
Column:	Waters XTerra™ MS C_{18}, 2.1 x 100 mm, 3.5 µm
Mobile Phase:	20% Acetonitrile / 10 mM Buffer NH_4HCO_3, pH 9.0
Flow Rate:	200 µL/min; flow split 1:1 pre-detector
Inject Volume:	5 µL
PDA:	200 to 400 nm; monitor 288 nm
Mass Spec:	Electrospray positive mode (ESP)
SIR 1:	MW = 192, Cone @ 25V from 0 to 6.5 mins
SIR 2:	MW = 202, Cone @ 35V from 6 to 15 mins
Sample cleanup	Oasis® MCX Solid phase extraction cartridges

Sample Cleanup by Solid Phase Extraction

Solid phase extraction (SPE) was used to remove matrices from the samples and to concentrate the analytes, permitting detection of low levels. This SPE procedure is mixed-mode cation-exchange extraction followed by reversed-phase cleanup *using a single cartridge.* For basic analytes the 6 cc Oasis™ MCX cartridges can be used. However, different sample matrices require different cleanup procedures . Where there were high concentrations of interferences at high pH, e.g. in orange juice, the SPE cleanup included a wash with HCl. Where there were high concentration of acidic interferences, e.g. apple juice, the cleanup included a wash with NH_4OH, Then the analytes were eluted with methanol containing 4% NH_4OH. Solid phase extraction can provide sample cleanup and a 100X concentration of the analytes.

Choosing the Best Mobile Phase Buffer

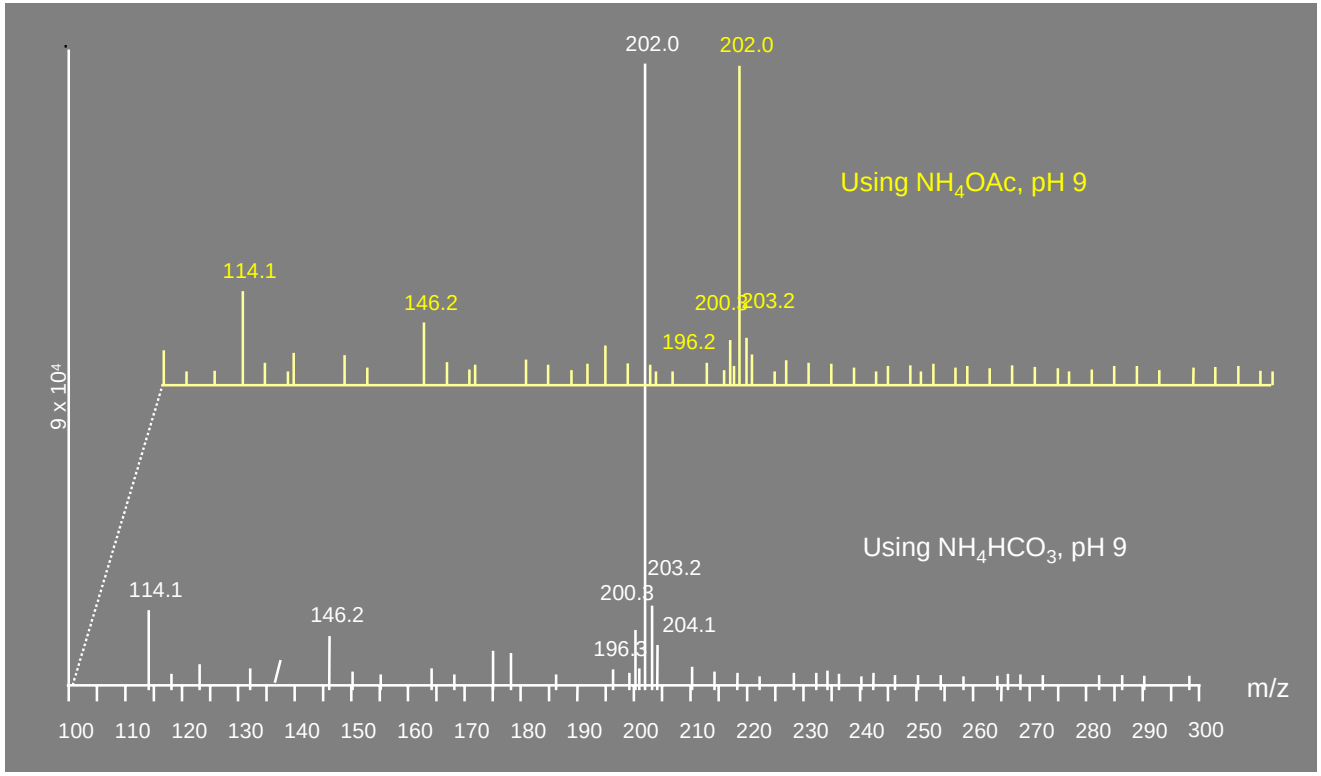


Figure 1: Mass Spectra in Different Mobile Phases

The correct choice of buffer can enhance the sensitivity of mass spectral detection. The infusion of 500 ppb of thiabendazole in two different buffers at pH 9.0 are shown. There was a 2X increase in the response when ammonium bicarbonate (lower spectrum) was used instead of ammonium acetate (upper spectrum), both at pH 9.

Figure 2: Grape Juice UV Chromatogram at 288 nm

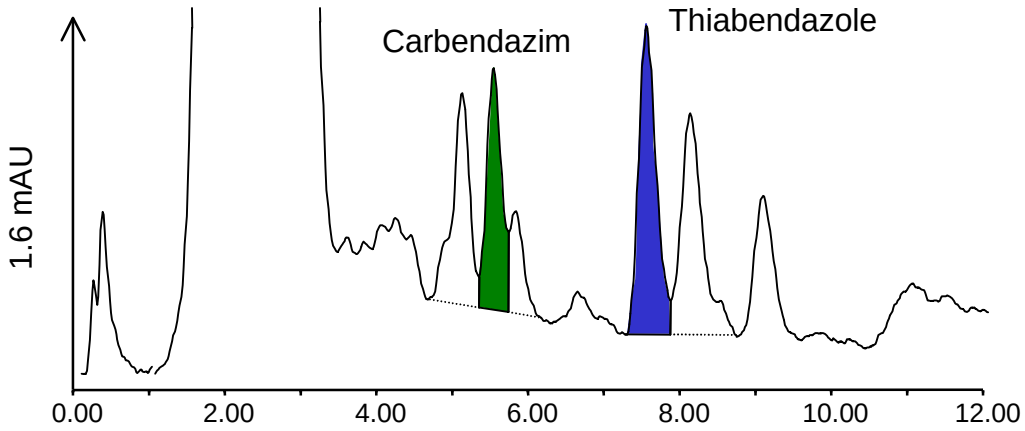
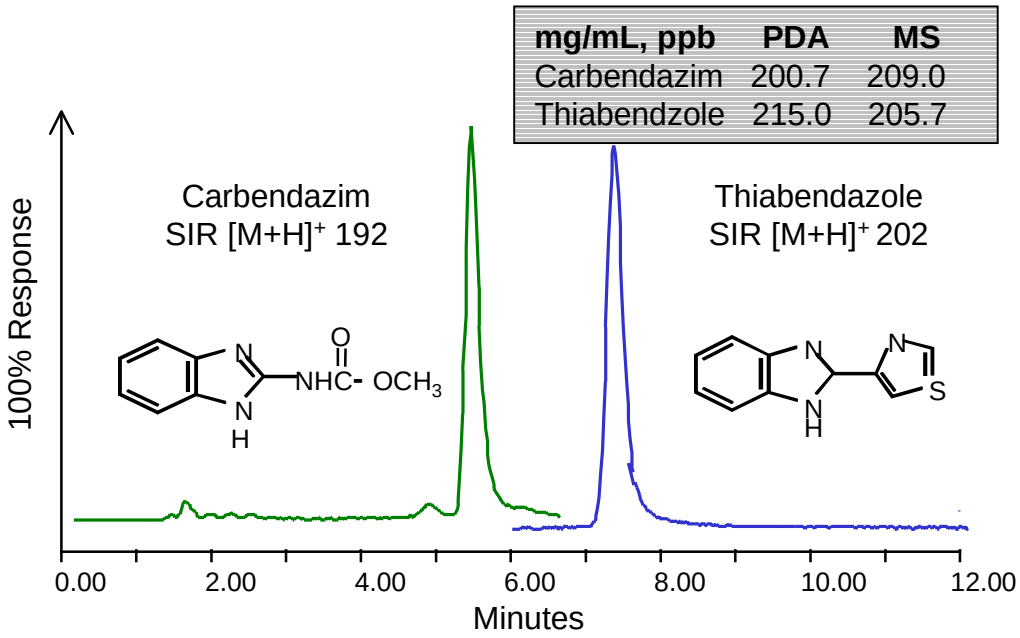


Figure 3: Grape Juice Mass Chromatograms at m/z 192 and 202



HPLC method. In other experiments the HPLC separation of carbendazim and thiabendazole were optimized on a Waters XTerra™ MS C_{18} column at pH 9 using an ammonium bicarbonate buffer. The peak tailing was reduced as the pH increased which increased the peak height and the sensitivity of the method. Ammonium carbonate produced less baseline noise than ammonium acetate and a 2X response (Figure 1).

UV detection. 288 nm is a wavelength that both analytes have absorbance (Figure 1). At 200 ng/mL or 200 ppb, there is a good response of the analytes. However, many other components in the grape juice also absorb, making automation of peak integration difficult. In addition, UV spectra can be used to check for coeluting peaks.

MS detection. For maximum sensitivity in electrospray positive mode, single ion recording (SIR) for carbendazim $[M+H]^+$ 192 and thiabendazole $[M+H]^+$ 202 were used (Figure 2). SIR is very selective, along with retention times, this confirms the presence of the analytes. Only the molecular ions are monitored and the matrix is not detected. This greatly simplifies the chromatograms and makes peak integration and quantitation significantly easier. Use of both PDA and MS detection provide complementary analytical information, and is recommended.

