HPLC Determination Of Fungicide Residues In Fruit Juices Using Photo-Diode Array And Mass Spectrometric Detection

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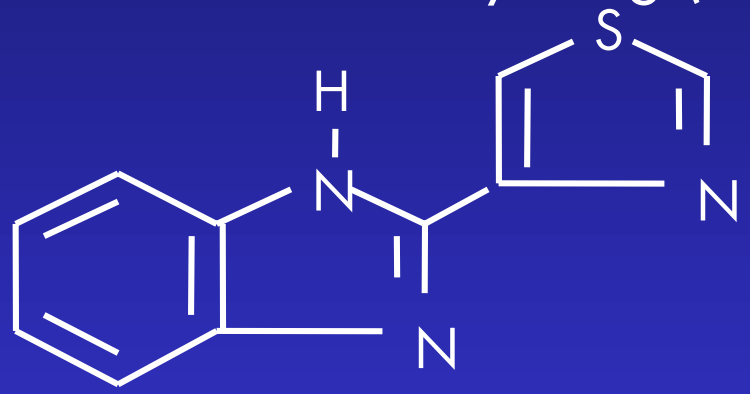
July 2000

Structures of Common Fungicides Thiabendazole and Carbendazim

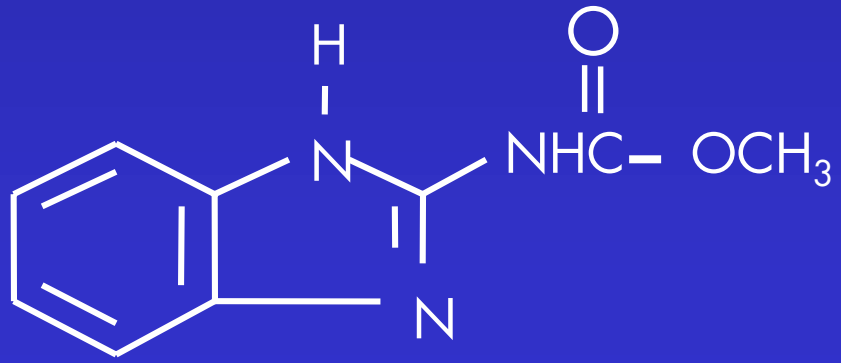
Systemic fungicide to control mold growth on fruits and vegetables; also used as a veterinary drug (nematocide)

Thiabendazole



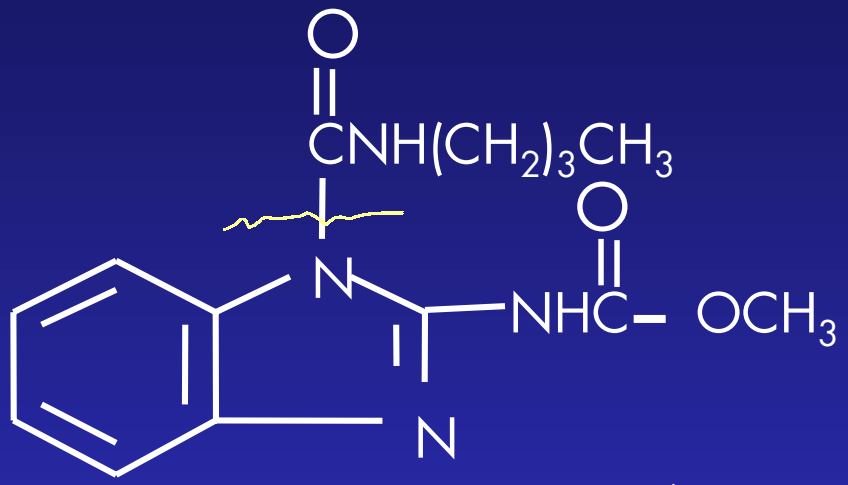
MW = 201

Carbendazim

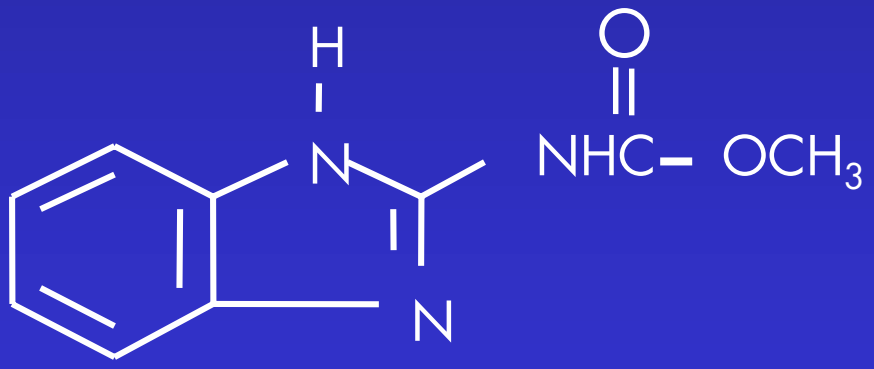


MW = 191

Degradation of Benomyl to Carbendazim

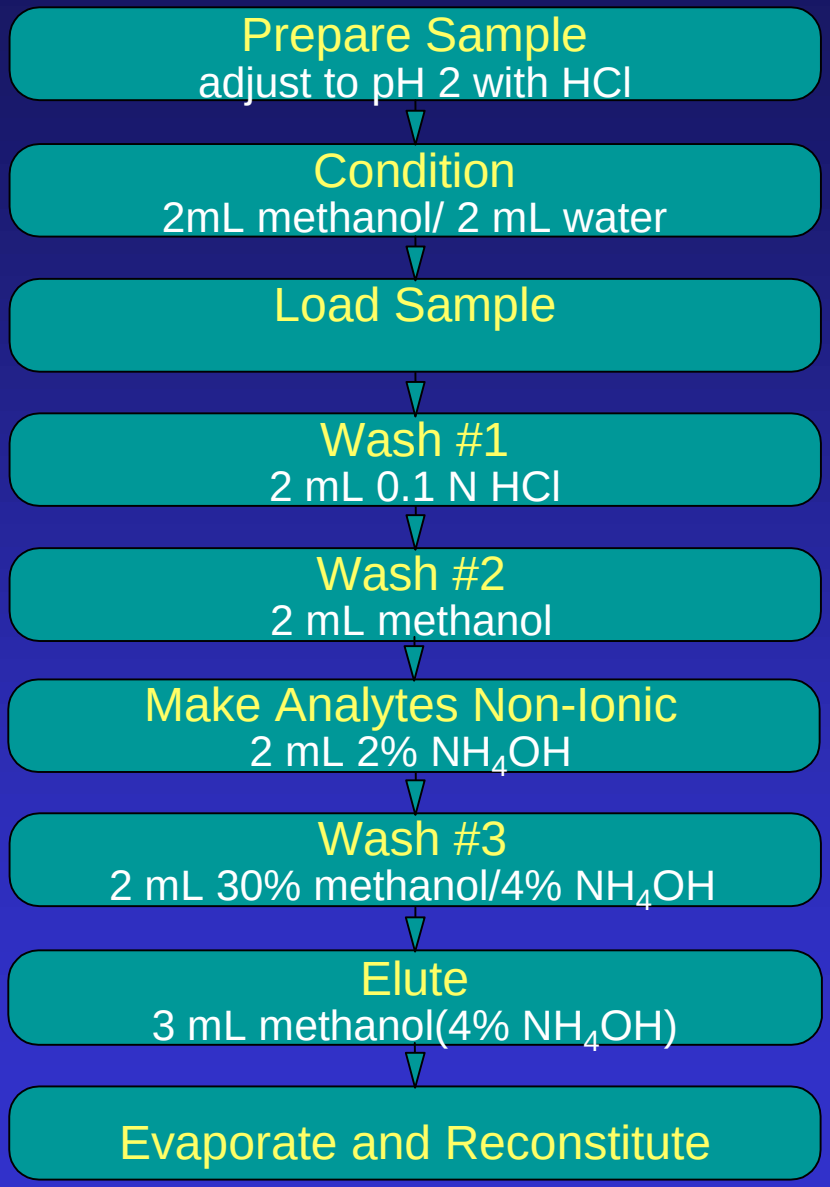


Benomyl



Carbendazim

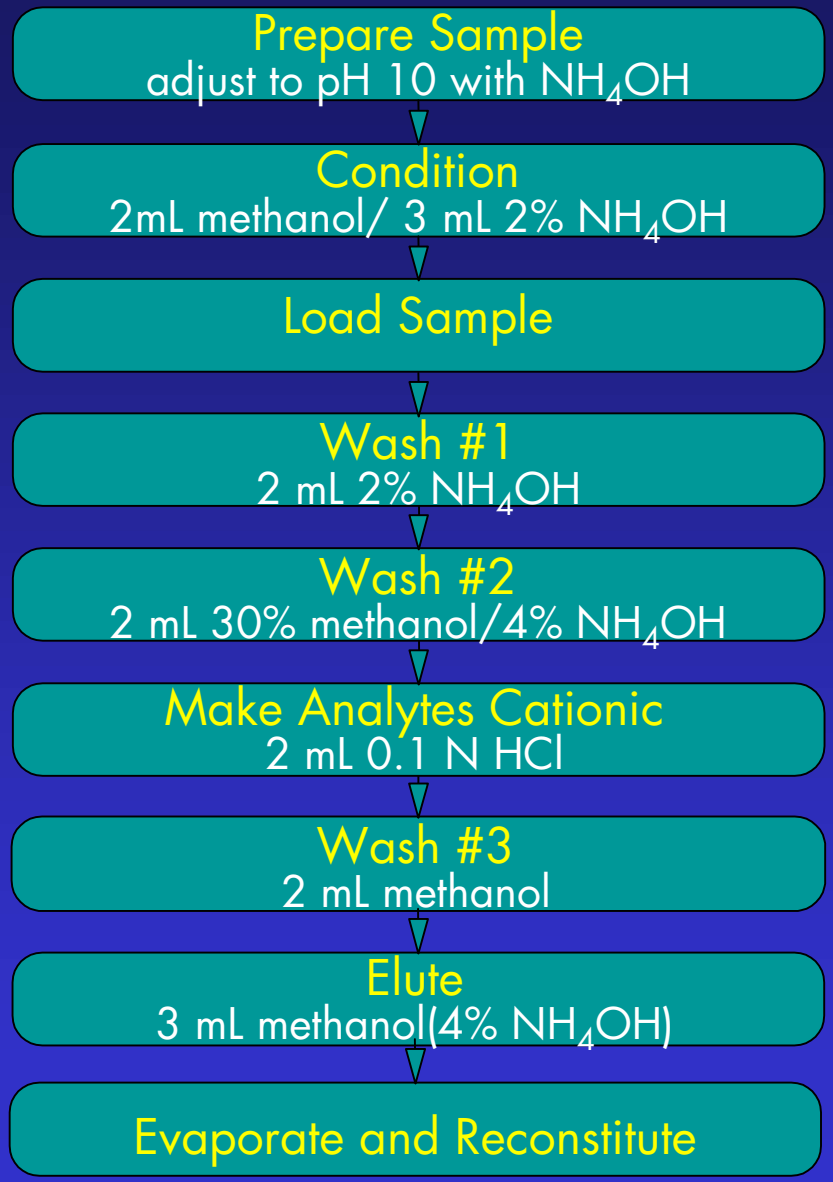
Oasis® MCX SPE Method A



- For Basic Analytes in Citrus juices (6 cc cartridge)
- This SPE procedure is mixed-mode cation-exchange extraction followed by reversed-phase cleanup

  Using a Single Cartridge
- This protocol provides high recovery of basic compounds in matrices, such as orange juice, that contain high concentrations of interferences at high pH

Oasis® MCX SPE Method B



- For Basic Analytes in Apple and Grape Juices (6 cc cartridge)
- This SPE procedure is reverse -phase extraction followed by cation-exchange cleanup
- Because the Oasis ® MCX sorbent is mixed mode, these sequential steps are performed

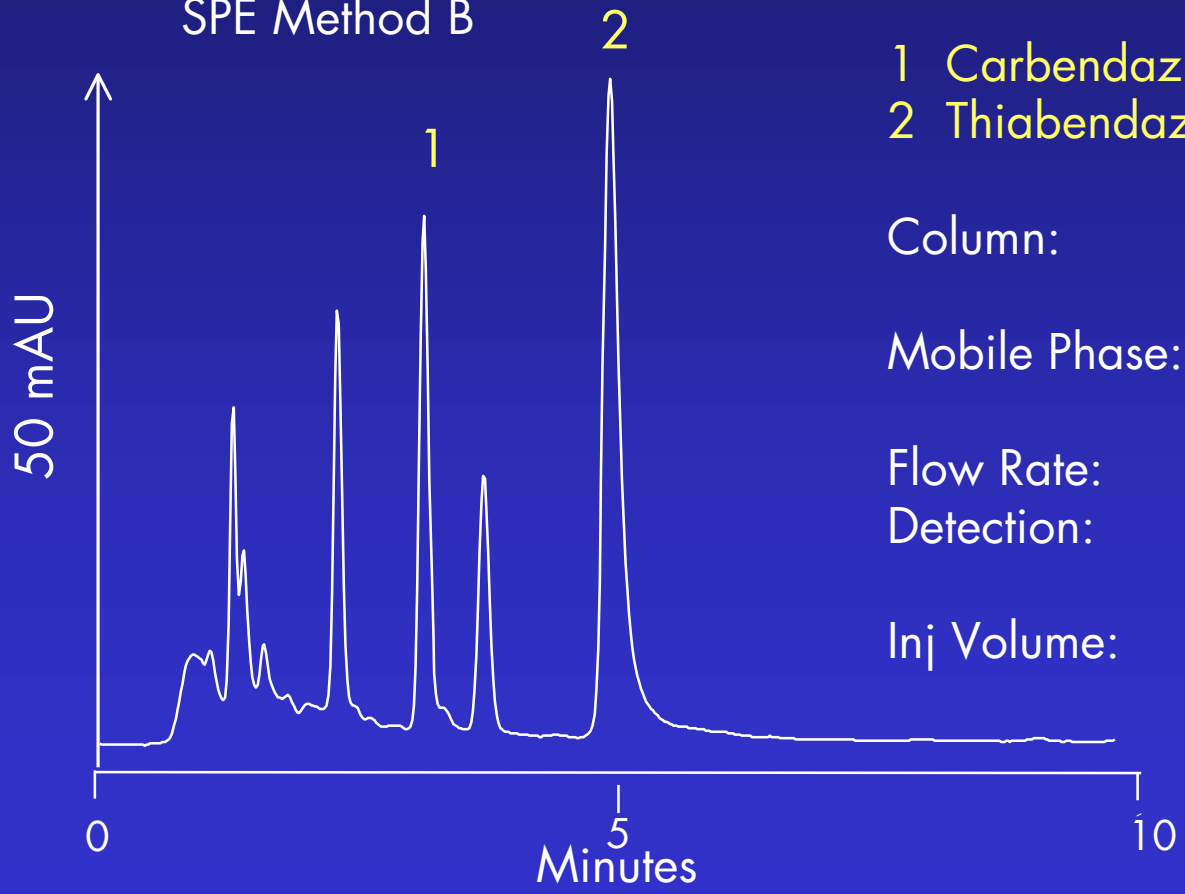
  Using One Cartridge
- This protocol provides high recovery of basic compounds in matrices, such as grape juice, that contain high concentrations of acidic interferences

Oasis® MCX SPE
Mixed-Mode Extraction and Clean-up of Juice Samples

- Provides sample enrichment
  - → up to 100 fold concentration using 6 cc cartridge
- Removes interferences
  - → improved chromatography
  - → provides longer column life
  - → reduces MS sample matrix effects (enhancement/suppression)
  - → reduces "fouling" of MS inlet cones

LC/PDA Determination of Fungicides
Chromatographic Conditions

10 mL of Commercial Apple Cider
OASIS® MCX Cartridge
SPE Method B



J.AOAC Int.
In Press

1 Carbendazim = 70 ppb
2 Thiabendazole = 130 ppb

Column:	XTerra™ RP18, 4.6 x 100 mm, 3.5 µm
Mobile Phase:	72% 20 mM Phosphate, pH 6.8 28% Acetonitrile
Flow Rate:	1 mL/min
Detection:	PDA Scan, 200 to 400 nm Extracted @288 nm
Inj Volume:	20 µL

Analysis of Carbendazim &Thiabendazole Using PDA and MS

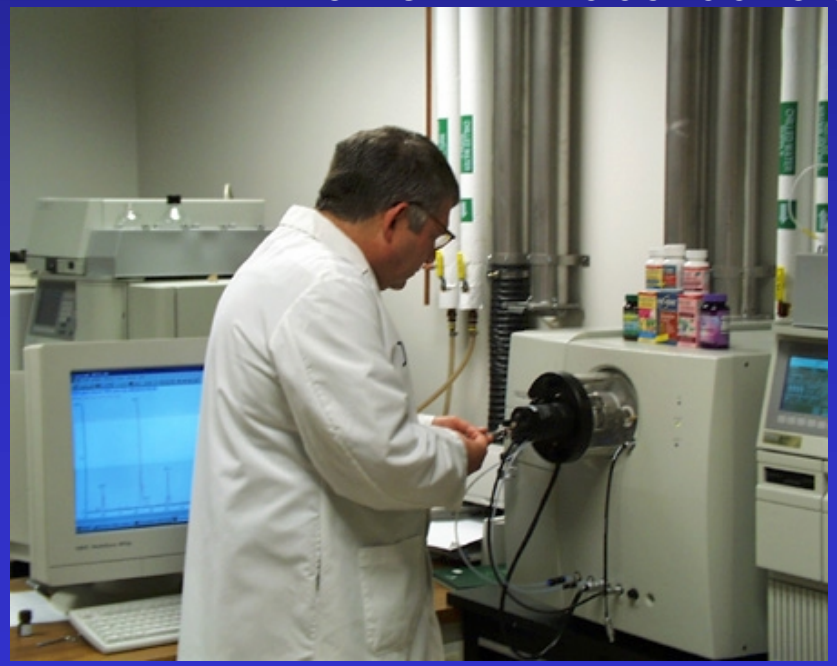
Modified Chromatographic Conditions for Electrospray MS Detection

HPLC:	Waters Alliance® System
Column:	Waters XTerra™ MS C_{18}, 2.1 x 100 mm, 3.5 µm
Mobile Phase:	80% 10 mM NH_4HCO_3 Buffer (pH 9)
	pH Adjusted with either NH_4OH or HOAc /20% AcCN
Flow Rate:	200 µL/min; Split flow ~50/50 pre-Detector
Inj Volume:	5 µL
PDA:	Scan 200 to 400 nm; Extracted @ 288 nm

MS Conditions

Instrument: Waters ZMD Zspray™ Mass Detector
Interface: Positive Electrospray (ESI^+)
Source heater: 125° C
Scan Function: Multiple Selected-Ion Recording (SIR)

SIR Group	Time mins.	Compound	Mass	Cone Voltage	Dwell Time
1	0-6.5	Carbendazim	192.1	25V	1.0 secs
2	6-15	Thiabendazole	202.0	35V	1.0 secs



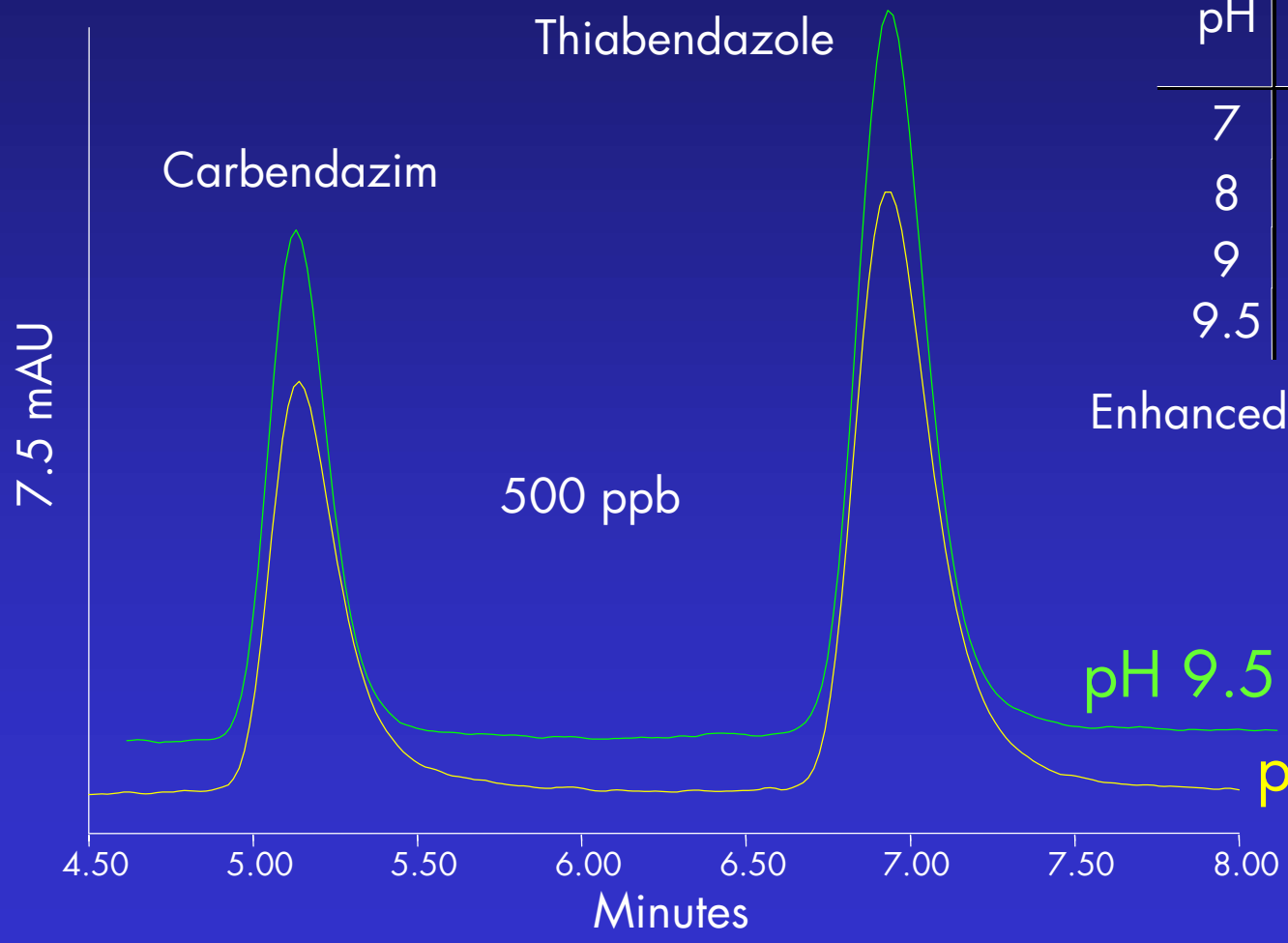
Mass Spec Control and Processing
Using Mass Lynx™

Choice of Mobile Phase pH and Buffer for Electrospray Mass Spectrometric Detection

- Ammonium acetate or formate, are the classic volatile buffers for MS detection
  - Natural pH about 7; may be adjusted with NH_4OH for alkaline pH but little buffering capacity
- Alkaline mobile phases absorb CO_2 from the atmosphere
  - Changes may occur in pH and composition
- Ammonium Bicarbonate has a natural pH of 8.4
  - Highly volatile; decomposes at 60°C, good buffering capacity to pH 10
  - Minimizes CO_2 absorption

Peak Tailing vs Mobile Phase pH

Xterra Using NH_4HCO_3



pH	USP Tailing Factor	Peak Height X 10^4
7	1.4818	5.49
8	1.3807	5.20
9	1.2806	5.52
9.5	1.2546	6.48

Enhanced peak symmetry and peak height with increasing pH

XTerra™ MS C_{18} column stable performance and long column life ***at high pH***

Chemistry of Ammonium Bicarbonate

In Solution

$$NH_4HCO_3 + H_2O \longrightarrow NH_4^+ + HCO_3^- + H_2O$$

pK_a of $NH_4^+ \longrightarrow NH_3 + H^+$ is 9.24

pKa of $HCO_3 + H^+ \longrightarrow H_2CO_3$ is 6.4

pKa of $HCO_3^- \longrightarrow H^+ + CO_3^{-2}$ is 10.3

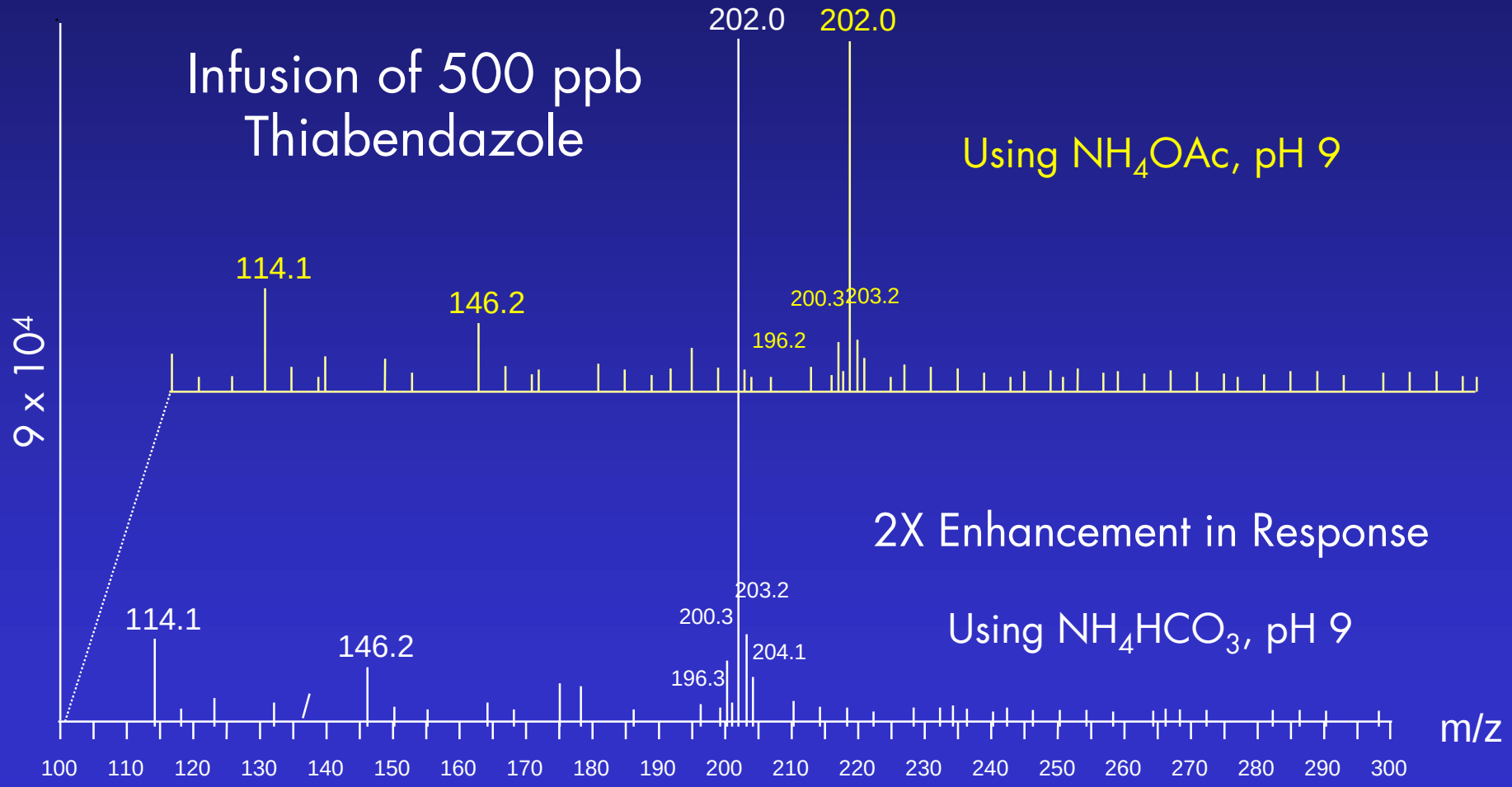
Buffering Range 6.4 to 10.3

pH of a 10 mM Solution is 8 to 8.2

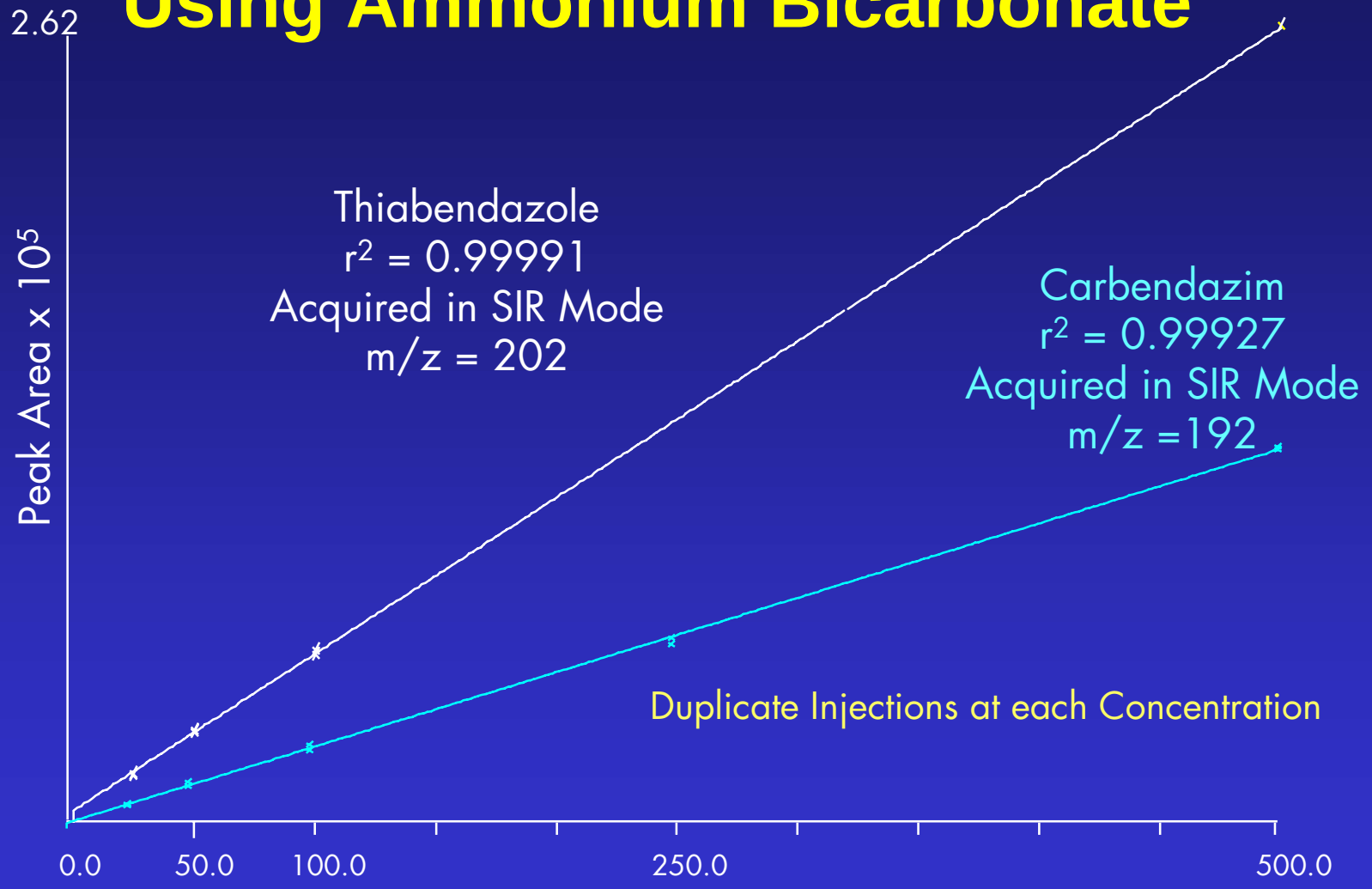
At the MS Interface

$$NH_4HCO_3 \xrightarrow{>60°C} NH_3\uparrow + CO_2\uparrow + H_2O$$

Choice of Buffer Type
Effect on Response



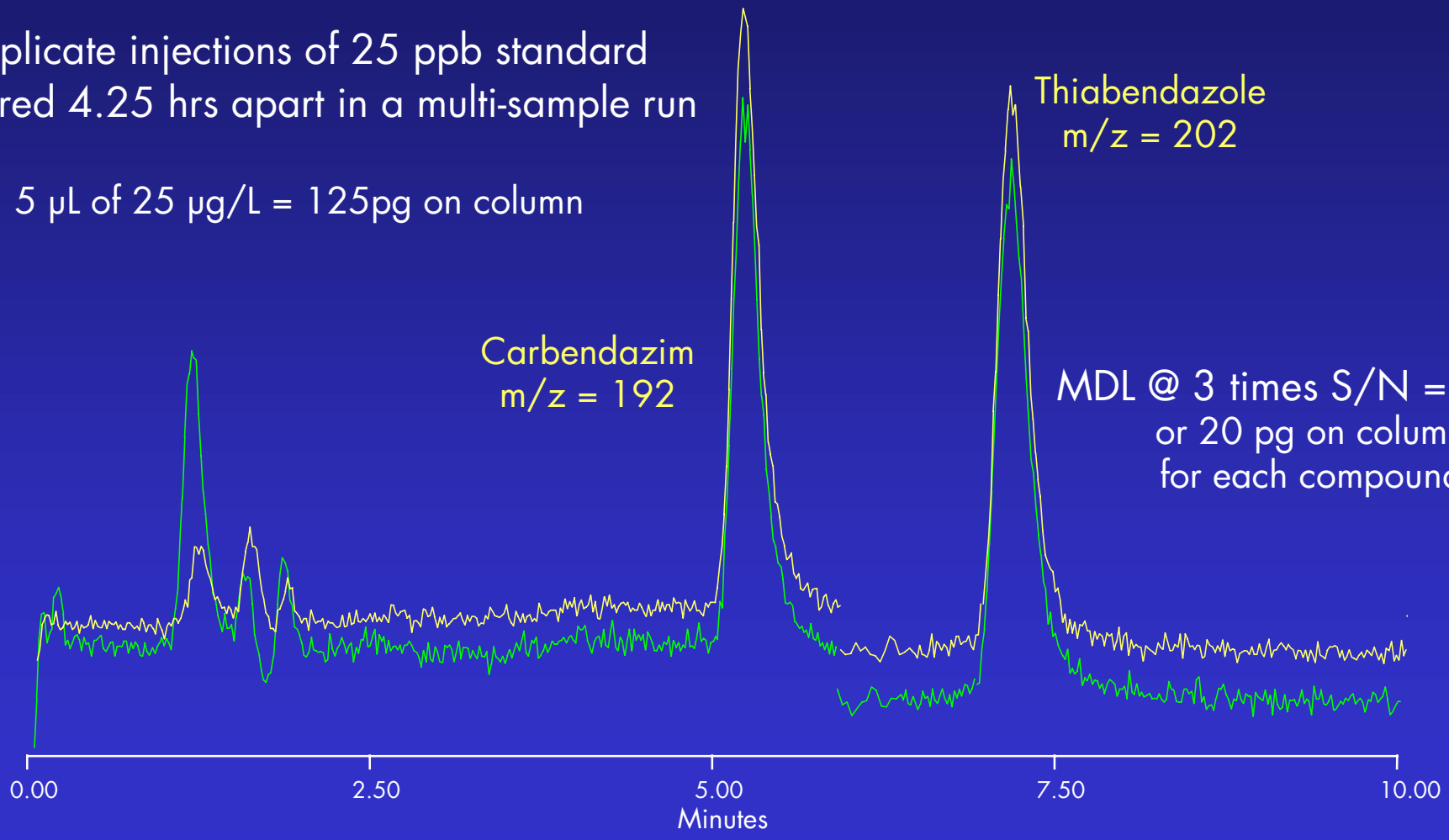
Mass Spec Linearity; 25-500 ppb
Using Ammonium Bicarbonate



MS Reproducibility and Detection Limits

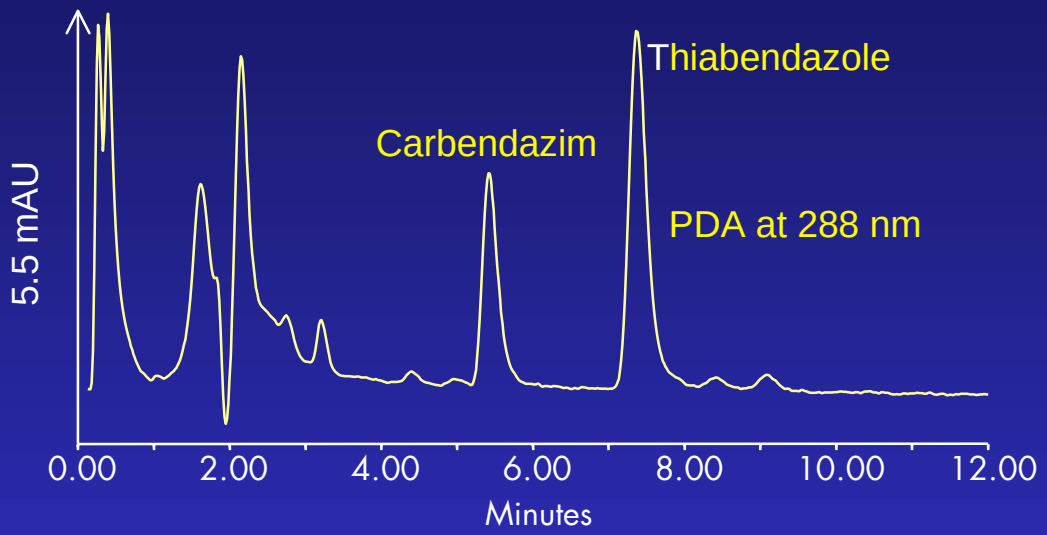
Duplicate injections of 25 ppb standard
acquired 4.25 hrs apart in a multi-sample run

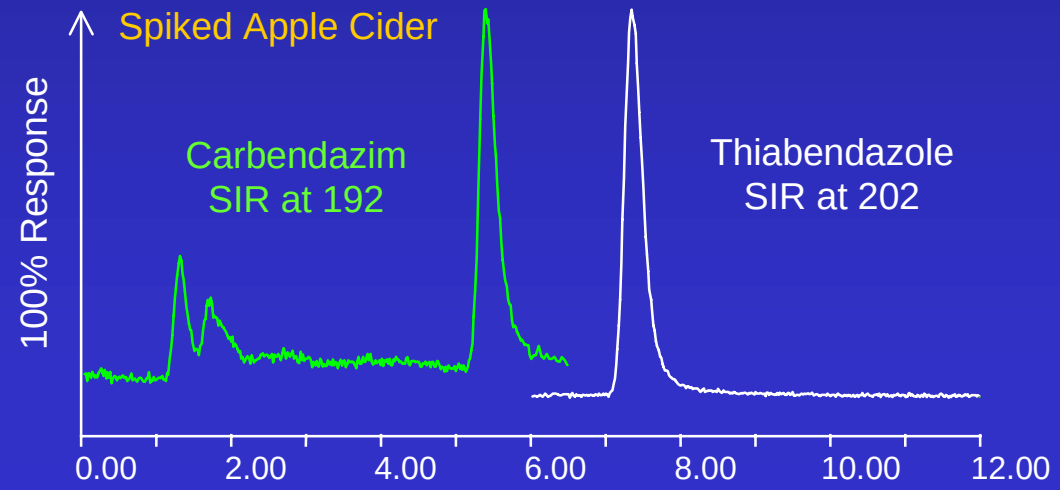
5 µL of 25 µg/L = 125pg on column



MDL @ 3 times S/N = 4 ppb
or 20 pg on column
for each compound

PDA vs MS
Quantitation Comparison





Carbendazim (ng/mL, ppb)

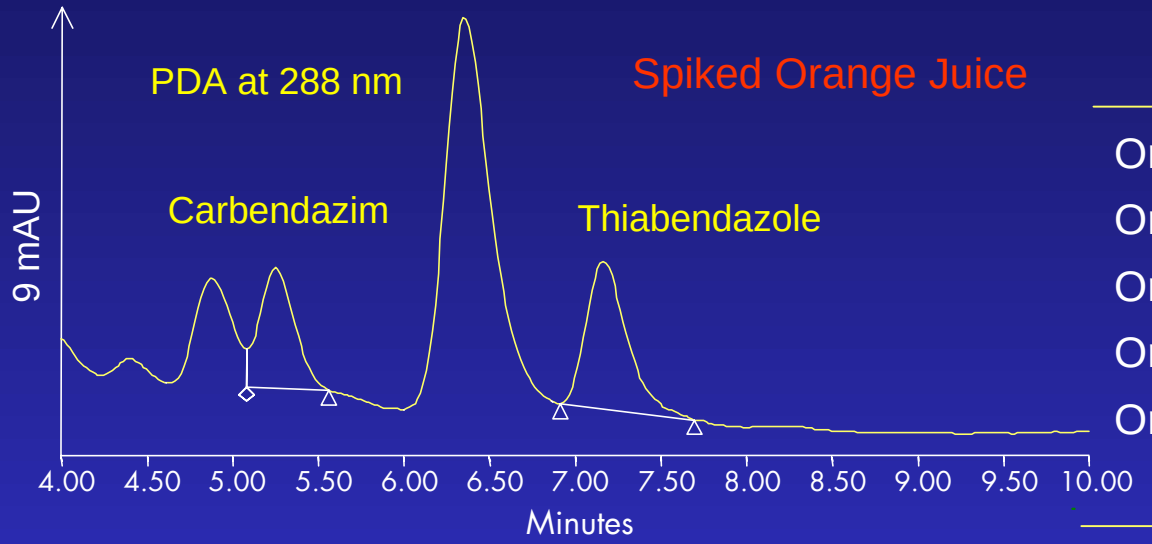
Sample*	PDA	MS
Apple Cider 1	376.6	392.3
Apple Cider 2	379.6	381.7
Apple Cider 3	387.4	391.3
Apple Cider 4	394.5	399.9
Apple Cider 5	388.1	406.7

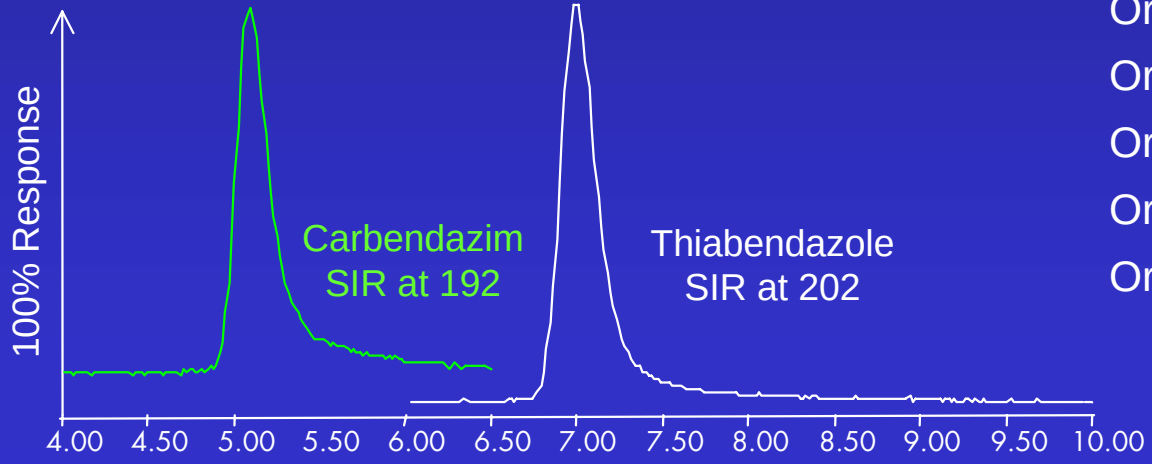
Thiabendazole (ng/mL, ppb)

Apple Cider 1	438.9	429.8
Apple Cider 2	425.0	419.5
Apple Cider 3	425.8	428.1
Apple Cider 4	455.9	443.9
Apple Cider 5	442.4	442.0

* Represents a 10X Concentration Factor
After Oasis MCX SPE Method B

PDA vs MS
Quantitation Comparison





Carbendazim (ng/mL, ppb)

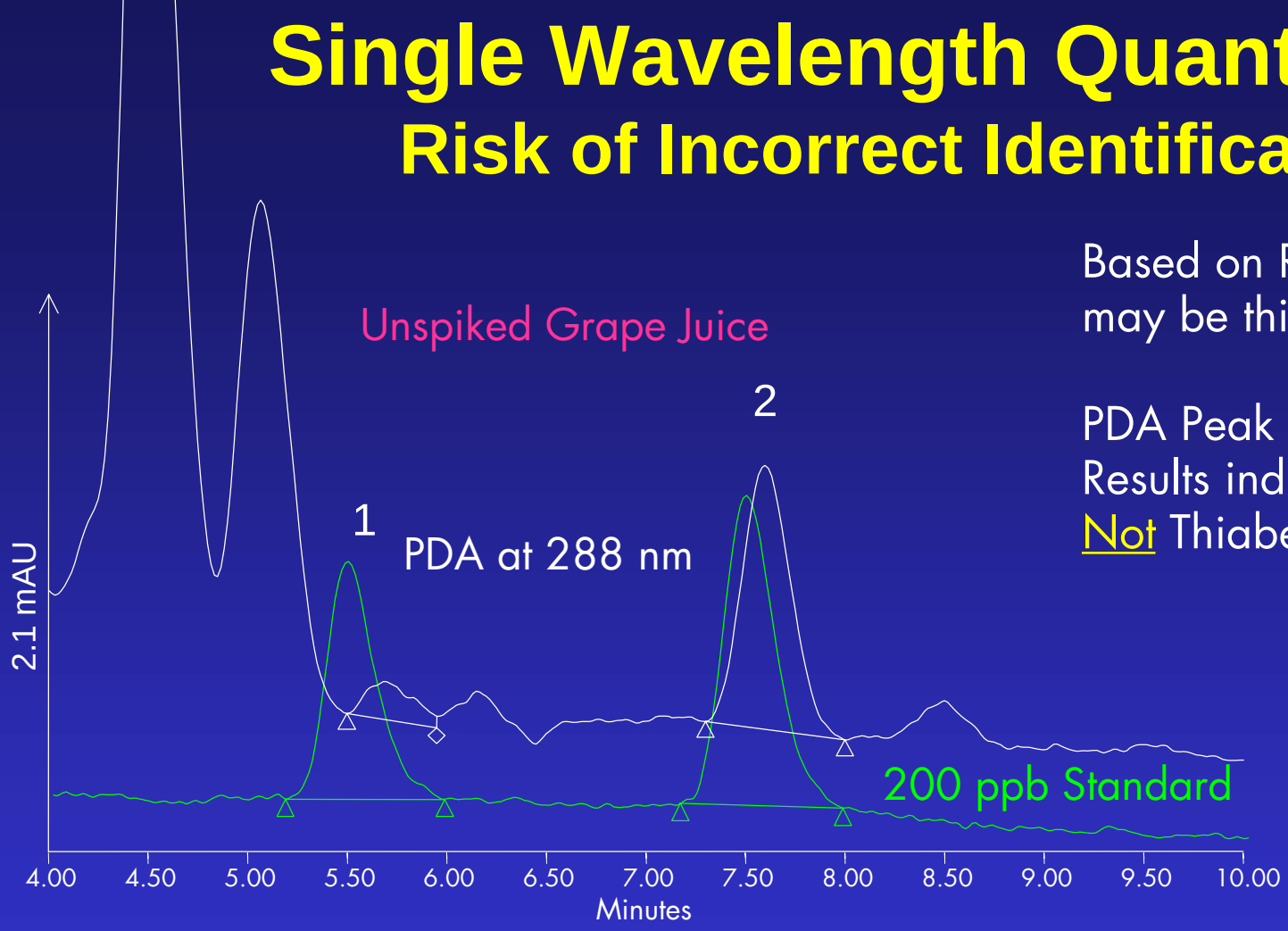
Sample*	PDA	MS
Orange Juice 1	452.7	524.1
Orange Juice 2	447.7	519.9
Orange Juice 3	446.4	522.1
Orange Juice 4	439.5	531.5
Orange Juice 5	440.4	516.0

Thiabendazole (ng/mL, ppb)

Orange Juice 1	453.3	455.4
Orange Juice 2	459.7	460.2
Orange Juice 3	435.1	440.0
Orange Juice 4	459.5	458.7
Orange Juice 5	465.0	464.8

* Represents a 10X Concentration Factor
After Oasis MCX SPE Method A

Single Wavelength Quantitation
Risk of Incorrect Identification



Based on Retention time, peak 2 may be thiabendazole

PDA Peak Purity and Peak Match Results indicate Peak 2 is Not Thiabendazole

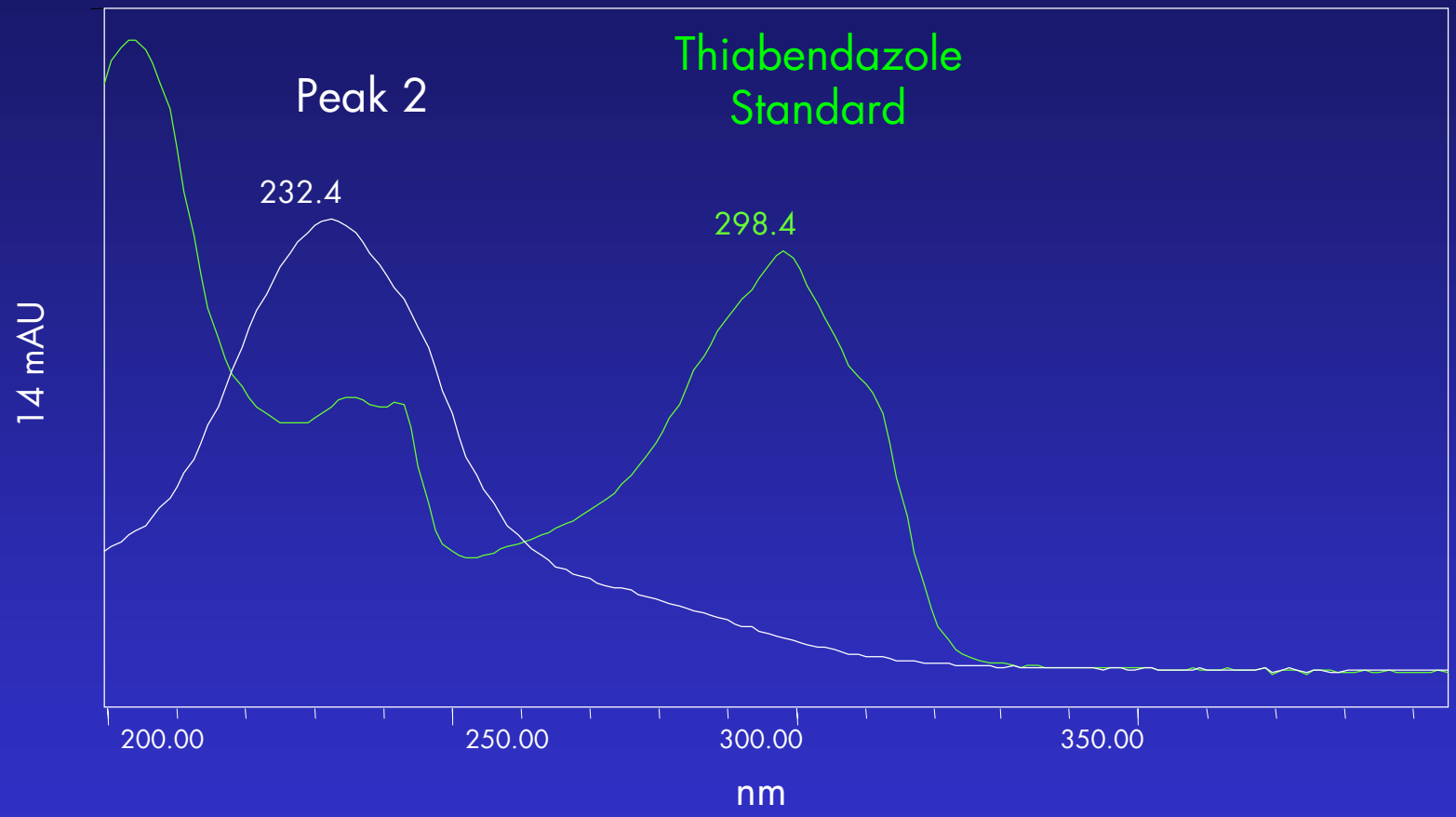
Questionable Results?

PDA Peak Results

	Analyte	Ret Time	Peak Area	Purity Angle	Purity Threshold	PDA Match Angle	PDA Match Threshold	Amount	Units
1	Carbendazim	5.689	2625	23.328	9.177			45.79	ppb
2	Thiabendazole	7.596	18330	1.050	0.402			170.58	ppb

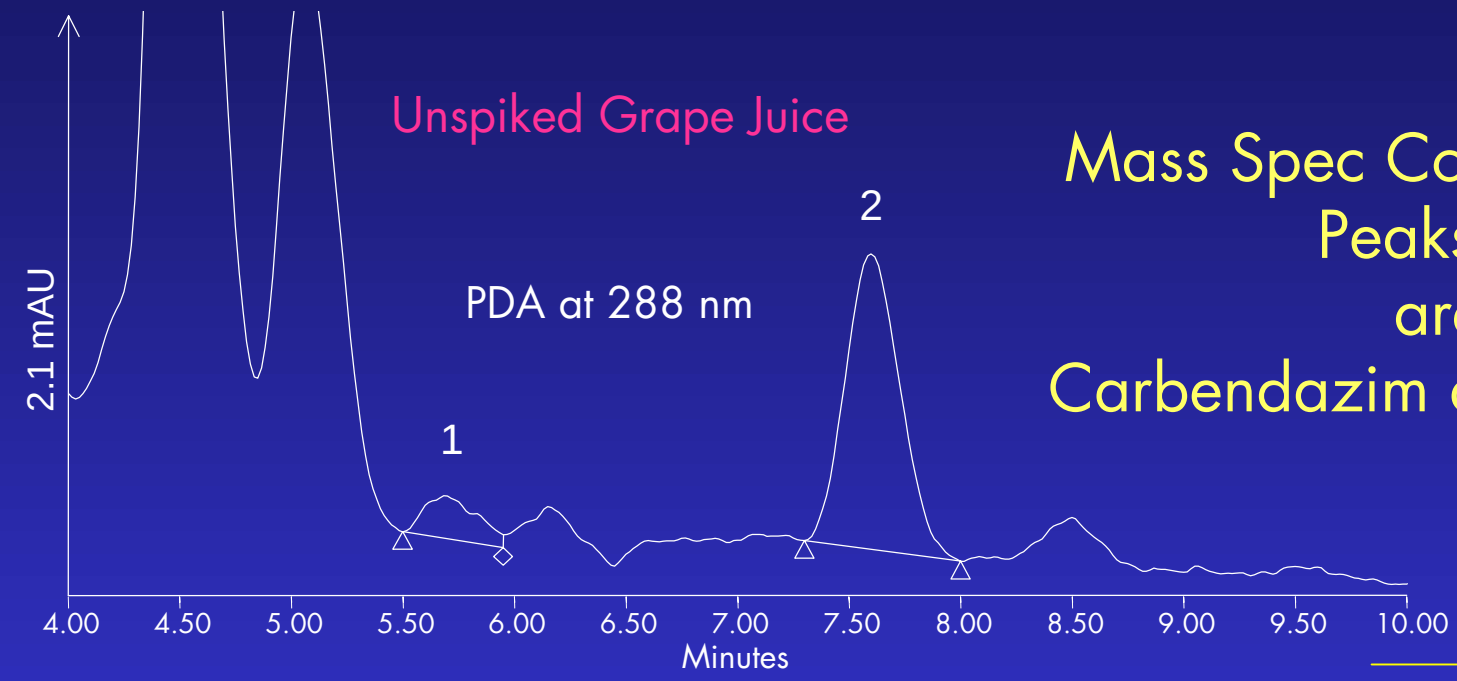
Single Wavelength Quantitation

Risk of Incorrect Identification



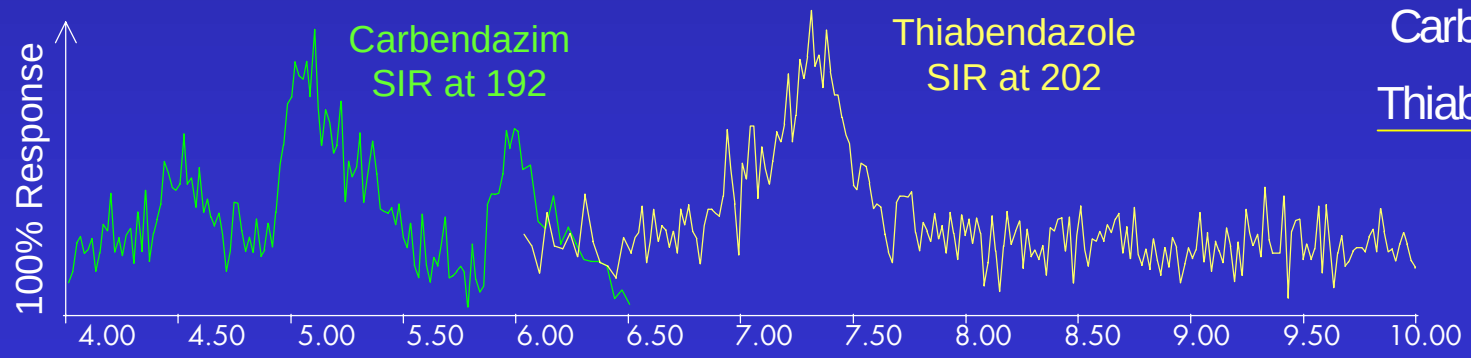
PDA Library Matching Indicates Different Spectra

Value of PDA and MS
Qualitative and Quantitative Confirmation

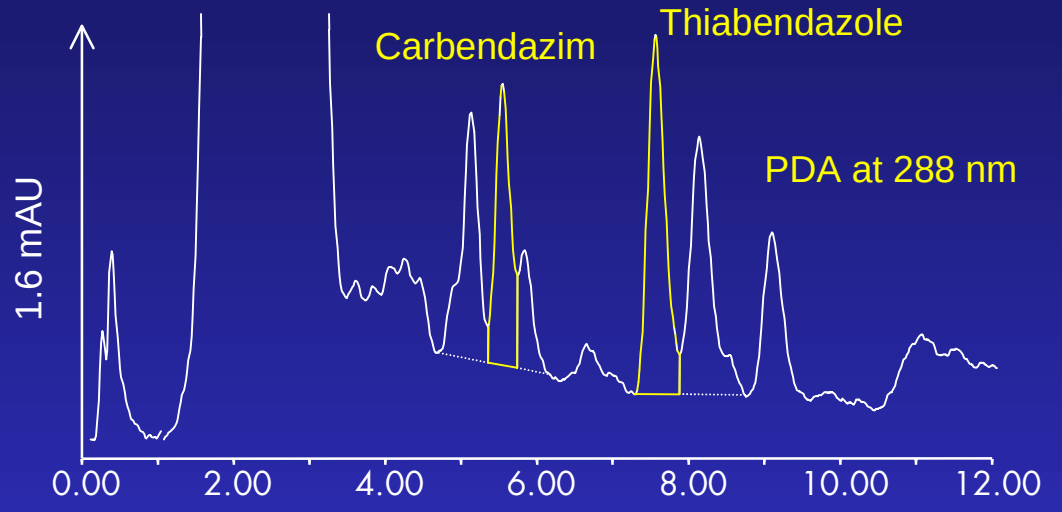


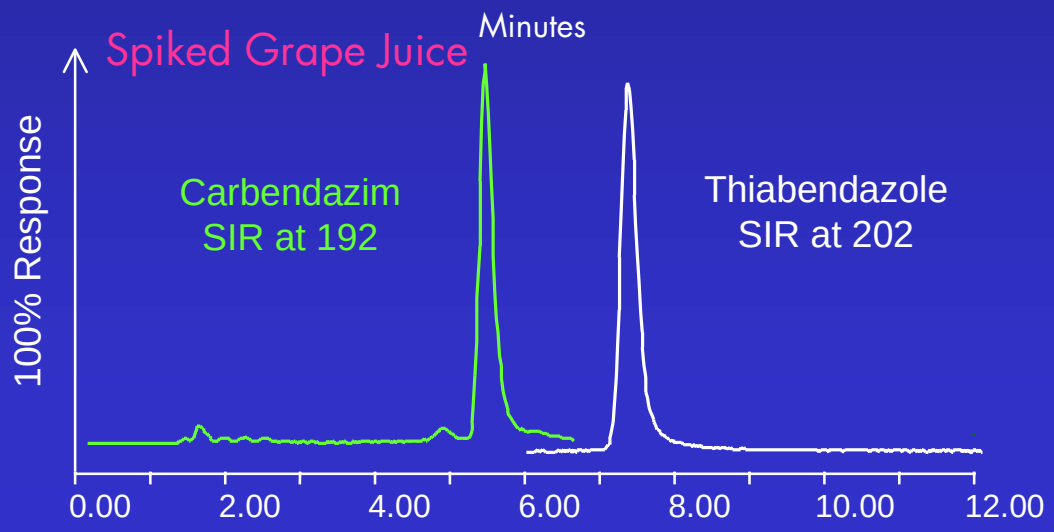
Mass Spec Confirms PDA Results
Peaks 1 and 2
are NOT
Carbendazim and Thiabendazole

	288 nm	Mass Spec
Carbendazim	45.8	ND
Thiabendazole	170.6	ND



PDA vs MS
Quantitation Comparison
Chromatographic and Integration Interference





PDA Required Manual Integration
for Reproducible Results

ng/mL, ppb	PDA	Mass Spec
Carbendazim	200.7	209.0
Thiabendazole	215.0	205.7

MS Confirms
PDA Results

Recovery of Fungicides From Juices
Summary of Oasis® MCX Extraction Data

	PDA		Mass Spec	
	Carbendazim	Thiabendazole	Carbendazim	Thiabendazole
Orange Juice	89% (2%RSD)	91% (3% RSD)	104% (1% RSD)	91% (2% RSD)
Apple Juice	87% (2% RSD)	98% (3% RSD)	89% (3% RSD)	99% (3% RSD)
Grape Juice	Interference		93% (9% RSD)	100% (6% RSD)

Spike level: 50 ng/mL for apple and orange, 20 ng/mL for grape

Summary

- A validated chromatographic method designed for a specific sample matrix, may not be appropriate for a different matrix. Apples vs Oranges vs Grapes
- There is a risk of incorrect analyte identification and quantitation when using only a single wavelength UV detection
- Use of PDA peak purity and library spectra matching aids in analyte identification and the possibility of biased quantitation
- Mass Spectrometry's SIR specificity confirms analyte identification and quantitation

Summary

- Oasis® MCX SPE procedures developed for LC/UV analysis were successfully employed for LC/MS analysis
- LC/UV methods may require modifications for use with Mass Spec detection; eliminate non-volatile buffers
- Ammonium bicarbonate is an excellent buffer choice for alkaline pH (8 to 10) work
- Can do positive electrospray at alkaline pH
- Use of alkaline pH with Waters XTerra™ columns extends chromatographic options