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TMR0812B

AZOXYSTROBIN: DETERMINATION OF AZOXYSTROBIN AND R230310 IN CROPS BY GAS CHROMATOGRAPHY WITH NITROGEN-PHOSPHORUS DETECTION (WRC-98-114)

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Report Title

Azoxystrobin:
Determination of Azoxystrobin and R230310 in Crops by Gas Chromatography with Nitrogen-Phosphorus Detection

Author

Cynthia Lipton

Report Issue Date

August 12, 1998

Performing Laboratory

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Richmond, CA 94804-4610

Report Number

TMR0812B

Certification of Authenticity

I, the undersigned, declare that this study was performed under my direction and that this report represents a true and accurate record of the results obtained.

Cynthia R. Lipton — Aug 11, 1998

Cynthia R. Lipton
Senior Research Technician
Zeneca Inc.

Date

Approved By:

Merle Kleinschmidt — Aug 11, 1998

Merle Kleinschmidt
Laboratory Lead
Dietary Exposure
Zeneca Inc.

Date

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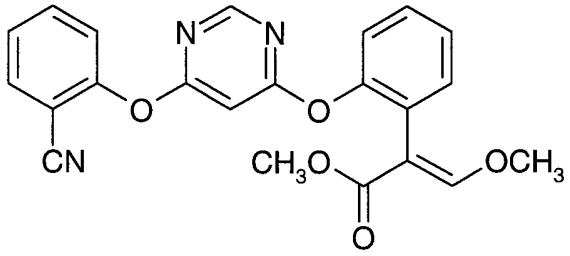
Determination of Azoxystrobin and R230310 in Crops by Gas Chromatography with Nitrogen-Phosphorus Detection

Author: Cynthia R. Lipton

1 Scope

This method is based on RAM 243/05 and is intended for the determination of residues of azoxystrobin (also known as ICIA5504) and its Z-isomer, R230310 in crops. Chemical structures and names for these compounds are given below.

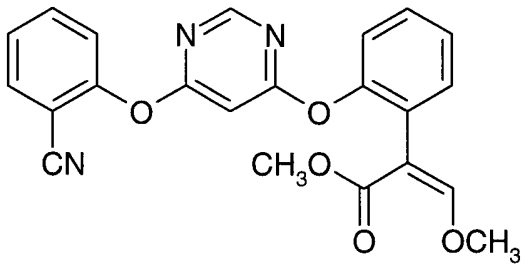


azoxystrobin

IUPAC Chemical Name:

Methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate

CAS Registration number: 131860-33-8.



R230310

Methyl (*Z*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate

The limit of quantitation for this method is 0.01 mg/kg of azoxystrobin and R230310 in crops.

2 Summary

Prepared crop samples are extracted with acetonitrile/water and filtered under vacuum. The analytes are then partitioned into dichloromethane and the dichloromethane fraction is evaporated to dryness. The residuum containing the analytes is then redissolved in 20:80 ethyl acetate: hexane and cleaned up on a florisil column. Azoxystrobin and R230310 residues are determined by gas chromatography with nitrogen-phosphorus detection using temperature programmable on-column injection.

3 Analytical Procedure

3.1 Extraction

Weigh a representative 10.0 g sample of prepared crop into a pint mason jar or blender jar. If appropriate, fortify untreated control samples with known amounts of azoxystrobin and R230310 at this time. Add 100 mL 90:10 (v:v) acetonitrile:water to the jar and blend for 5 minutes at medium speed. Using a Buchner funnel and minimal vacuum, filter the extract through Whatman #1 filter paper into filter flask or a 250 mL flat-bottom boiling flask. Wash the jar and filter paper with an additional 40 mL of acetonitrile. Pour the sample extract into a graduated cylinder and add enough acetonitrile to adjust the volume to 150 mL.

3.2 Fortifications

If available, analyze fortified and unfortified untreated control samples with each sample set to demonstrate method recovery and performance. Prepare fortified controls by adding known amounts of azoxystrobin and R230310 to the untreated control samples before extraction. Extract the sample as detailed above.

3.3 Dichloromethane Partition

Transfer a 15 mL aliquot (equivalent to 1 g of crop) into a 250 mL separatory funnel. Add 7.5 mL of 5% NaCl solution to the separatory funnel. Add 15 mL dichloromethane to the funnel, shake for 5 seconds and vent the separatory funnel. Shake for 30 seconds and allow the phases to separate. Filter the lower dichloromethane phase through a glass funnel plugged with glass wool containing sodium sulfate (prewashed with dichloromethane) into a 100 mL flat-bottom boiling flask. Rinse the sodium sulfate with 10 mL dichloromethane and evaporate the contents of the flask to dryness on a rotary evaporator.

3.4 Florisil Column Chromatography

Condition the florisil column (Fisher Prepsep, P466R) with 5 mL methanol, followed by 10 mL 20:80 (v/v) ethyl acetate:hexane by gravity flow. Allow each

solvent to reach the column bed before adding the next. Discard the conditioning solvents. Redissolve the residuum in the flask in 2 mL 20:80 (v/v) ethyl acetate:hexane and quantitatively transfer the contents of the flask to the florisil column using a Pasteur pipette. Add 8 mL 20:80 (v/v) ethyl acetate:hexane to the flask and transfer it onto the column. Discard these washes. Elute the azoxystrobin and R230310 by adding 20 mL 70:30 (v/v) ethyl acetate:hexane to the column. Collect this fraction in a 100 mL flat bottom boiling flask and evaporate the solvent to dryness on a rotary evaporator. Redissolve the residuum in 1.0 mL toluene for gas chromatographic analysis.

Alternate Florisil Cleanup

If the above cleanup procedure is not suitable for the particular crop being analyzed, i.e. low recovery or interfering coextractive peaks, try this alternate florisil cleanup. Prepare a 100 g batch of 5% water deactivated florisil (100-200 mesh) by drying overnight in an oven at 110°C. Allow the florisil to cool and add 5 x 1 mL of deionized water. Tumble for 10 minutes between each milliliter of water to ensure thorough mixing. Place a glass wool plug in the bottom of a 10 mm diameter glass chromatographic column. Add 2 g 5% deactivated florisil to the column. Condition the column with 15 mL hexane. Cap the column with about 0.5 g sodium sulfate. Allow the conditioning solvent to reach the column bed before loading the sample extract. Redissolve the residuum in the flask in 2 mL 20:80 (v/v) ethyl acetate:hexane and quantitatively transfer the contents of the flask to the florisil column using a Pasteur pipette.

Wash the column with 20 mL 30:70 ethyl acetate:hexane. Discard the wash. Elute the azoxystrobin and R230310 by adding 20 mL 70:30 (v/v) ethyl acetate:hexane to the column. Collect this fraction in a 100 mL flat bottom boiling flask and evaporate the solvent to dryness. Redissolve the residuum in 1.0 mL toluene for gas chromatographic analysis.

Note: To determine the elution pattern of the analytes in a particular sample matrix, fortify a control extract with known amounts of the analytes. Condition and load the sample on the column as described above. Discard the conditioning, loading and washing solvents. Collect 3 separate 10 mL fractions of the 70:30 (v/v) ethyl acetate:hexane elution solvent. Follow this by collecting a separate 5 mL fraction of the same solvent. Evaporate these to dryness and redissolve in 1 mL toluene and analyze each fraction separately.

4 Gas Chromatography with Nitrogen-Phosphorus Detection

4.1 Operating Conditions

GC:	Hewlett Packard 5890 Series II Plus or equivalent
Detector:	Nitrogen-phosphorus
Autosampler:	Hewlett Packard 7673 or equivalent
Column:	Restek RTX-200, 30 m x 0.53 mm x 1 µm film thickness
Injector:	On-column, temperature programmable injector
GC flow rates:	helium carrier flow rate = 20.2 mL/minute
	carrier + hydrogen = 23.2 mL/minute
	helium + auxillary + hydrogen = 28.6 mL/minute
	air = 88 mL/minute
Injector:	pressure = 13.9 psi, constant flow
	initial temperature = 90°C
	temperature rate = 150°C/minute
	final temperature = 270°C
	final time = 15 minutes
Oven:	initial temperature = 90°C
	initial time = 0.2 minute
	temperature rate = 30°C/minute
	final temperature = 270°C
	final time = 11 minutes
Expected Retention Times:	Azoxystrobin - 10.8 minutes, R230310 - 11.2 minutes

4.2 Calibration Procedures

Prior to calibration, condition the inlet and column with one or two injections of a crop extract.

Linearity can be established by injecting a range of standard concentrations. Preferably, calibration standards should be reinjected after every three or four sample extracts. Peaks are identified as azoxystrobin or R230310 based upon the coincidence of retention times of the unknown peaks with the bracketing calibration standards.

Quantitation is based upon the average of the response factors (concentration/peak height) of the standard calibration solutions bracketing the sample injections. The average response factors of the bracketing standards are used to calculate levels of azoxystrobin and R230310.

4.3 Interferences / Matrix Effect

No matrix effects have been observed.

4.4 Confirmatory Techniques

If it is necessary to confirm the identities of the peaks assigned as azoxystrobin and R230310, the confirmation can be achieved using a GC column of different polarity. The untreated control should be analyzed to demonstrate freedom from matrix interferences.

4.5 Time Required for Analysis

The analysis can be completed by one person in one 8-hour work day. The procedures within this method can be stopped at any point. The extracts can be retained at room temperature overnight.

4.6 Calculations

The concentration of the analyte in the original sample is calculated by using the external standard method. In this method, the response (peak height) obtained for the analyte resulting from injection of the sample extract is compared to the response obtained from separate injections of a known analyte (calibration solution). To use the calculations shown below, the injection volumes for all calibration solutions and sample extracts must be the same. The response of the standard injections before and after the samples of interest (bracket) is used to determine the calibration (average response) factor for those samples.

4.7 Calibration Factor

Calculate the calibration or average response factor, F, for injections of the same calibration solution as follows:

$$F = 2 \times \frac{C}{(R_1 + R_2)}$$

Where:
F = calibration or average response factor (µg/mL/peak height)
C = concentration of calibration solution (µg/mL)
R_1 = response units (e.g., peak height) for first calibration
R_2 = response units (e.g., peak height) for second calibration

4.8 Analyte in Sample

Calculate the analyte concentration, A, in the original sample as follows:

$$A = \frac{(F \times R)}{C}$$

Where:
A = concentration of analyte in original sample (µg/g)
F = calibration or average response factor (µg/mL/peak height)
R = sample response units from detector, peak height
C = concentration of sample in final extract (sample to solvent ratio, usually g/mL)

5 Control and Recovery Experiments

If available, at least one untreated or control sample should be analyzed with each sample set. This ensures that no unobserved contamination of the samples occurs prior to or during the analysis. At least one control sample should be fortified with azoxystrobin and R230310 in each sample set. Fortification levels should be based on anticipated residue levels.

6 Limit of Quantitation

The limit of determination or the limit of quantitation (LOQ) was assessed by carrying out recovery experiments at low fortification levels. In this laboratory, the LOQ has been established at 0.01 µg/g.

7 Limit of Detection

The limit of detection has not been established. It is below the limit of quantitation of 0.01 µg/g.

8 Method Validation Studies

8.1 Recovery Data - Precision and Accuracy

The range of recoveries of azoxystrobin and R230310 from fortified samples varied from 69 to 125%. The precision, as reflected in the % coefficient of variation (CV) values, varied from 5-20% (References 1, 2 and 3). Recovery data for almonds, wheat hay, grain and straw, and cucurbits are shown in the following tables.

Table 1. Recoveries of Azoxystrobin and R230310 from Almond Hull and Nutmeat

Sample Number	Commodity	Azoxystrobin		R230310	
		Fortification rate (mg/kg)	% Recovery	Fortification rate (mg/kg)	% Recovery
M301-10	Hull	0.01	119	0.01	106
M301-10	Hull	0.5	76	0.5	70
M301-10	Hull	3.0	86	3.0	84
M301-16	Nutmeat	0.01	115	0.01	110
M301-16	Nutmeat	0.1	94	0.1	75
Mean Recovery ± CV [A]:			98 ± 19%		89 ± 20%

[A] % CV = (standard deviation / mean) x 100

Table 2. Recoveries of Azoxystrobin and R230310 from Wheat Hay

Sample Number	Azoxystrobin		R230310	
	Fortification rate (mg/kg)	% Recovery	Fortification rate (mg/kg)	% Recovery
L509-1	10	92	10	83
L513-1	2	95	2	82
L501-101	1	90	1	91
L502-101	1	88	1	88
L504-1	1	106	1	108
L506-1	1	101	1	98
L515-1	0.5	114	0.5	82
L501-101	0.01	75	0.01	125
L512-1	0.01	116	0.01	108
Mean Recovery ± CV [A]:		97 ± 14%		96 ± 16%

[A] % CV = (standard deviation / mean) x 100

Table 3. Recoveries of Azoxystrobin and R230310 from Wheat Grain

Sample Number	Azoxystrobin		R230310	
	Fortification rate (mg/kg)	% Recovery	Fortification rate (mg/kg)	% Recovery
L503-7	0.05	96	0.05	85
L504-7	0.05	100	0.05	92
L506-7	0.05	117	0.05	119
L507-7	0.05	101	0.05	102
L510-7	0.05	108	0.05	101
L508-7	0.02	103	0.02	88
L512-7	0.02	118	0.02	79
L514-7	0.02	117	0.02	69
L501-110	0.01	92	0.01	76
L501-110	0.01	82	0.01	100
L502-110	0.01	91	0.01	84
L506-7	0.01	81	0.01	96
L507-7	0.01	82	0.01	88
L516-7	0.01	113	0.01	112
Mean Recovery ± CV [A]:		100 ± 13%		92 ± 14%

[A] % CV = (standard deviation / mean) x 100

Table 4. Recoveries of Azoxystrobin and R230310 from Wheat Straw

Sample Number	Azoxystrobin		R230310	
	Fortification rate (mg/kg)	% Recovery	Fortification rate (mg/kg)	% Recovery
I501-106	1	93	1	93
I506-4	1	113	1	103
I508-4	1	107	1	94
I512-4	1	116	1	115
I513-4	1	120	1	96
I502-106	0.5	99	0.5	102
I503-4	0.5	101	0.5	83
I505-4	0.5	109	0.5	93
I501-108	0.01	101	0.01	81
Mean Recovery ± CV [A]:		107 ± 9%		96 ± 10%

[A] % CV = (standard deviation / mean) x 100

Table 5. Recoveries of Azoxystrobin and R230310 from Cucurbits

Sample Number	Cucurbit Fruit	Azoxystrobin		R230310	
		Fortification rate (mg/kg)	% Recovery	Fortification rate (mg/kg)	% Recovery
M321-1	cantaloupe	0.1	106	0.1	90
M322-1	cantaloupe	0.3	99	0.3	91
M325-1	cantaloupe	0.1	97	0.1	85
M327-1	cucumber	0.1	100	0.1	88
M331-1	cucumber	0.1	94	0.1	89
M332-1	cucumber	0.01	120	0.01	92
M334-1	summer squash	0.1	102	0.1	95
M337-1	summer squash	0.2	113	0.2	100
Mean Recovery ± CV [A]:			104 ± 9%		91 ± 5%

[A] % CV = (standard deviation / mean) x 100

8.2 Linearity Statement

For acceptable quantitation of residue concentrations, all analyses should be carried out within the linear range of the detector response. For this method, the linear range is between 0.01 and 1.0 µg/mL azoxystrobin and R230310 in crops.

9 Examples of Typical Chromatograms

Examples of chromatograms are shown in Appendix 1 (from References 1, 2 and 3).

10 References

1. Roper, E.M.; Francis, P.D. and Lipton, C.R. (1997) *ICIA5504: Residue Levels on Almonds from Trials Conducted in the United States During 1996*, Zeneca Inc., Richmond, CA, Report No. RR 97-001B.

2. Roper, E.M.; Francis, P.D. and Storoni, H.R. (1996) *ICIA5504: Residue Levels on Wheat Hay Grain and Straw from Trials Conducted in the United States During 1995*, Zeneca Inc., Richmond, CA, Report No. RR 96-048B.

3. Roper, E.M.; Elvira, D.J.; Francis, P.D. and Lipton, C.R. (1997) *ICIA5504: Residue Levels on Cucurbits from Trials Conducted in the United States During 1996*, Zeneca Inc., Richmond, CA, Report No. RR 96-096B.

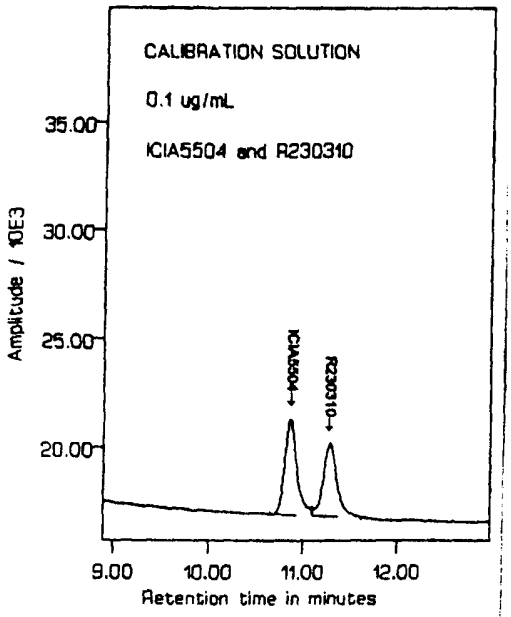
4. Burke, W.R. (1997) *Residue Analytical Method for the Analysis of Azoxystrobin and R230310 in Crops*, Zeneca Agrochemicals, SOP No. RAM 243/05.

Appendix 1

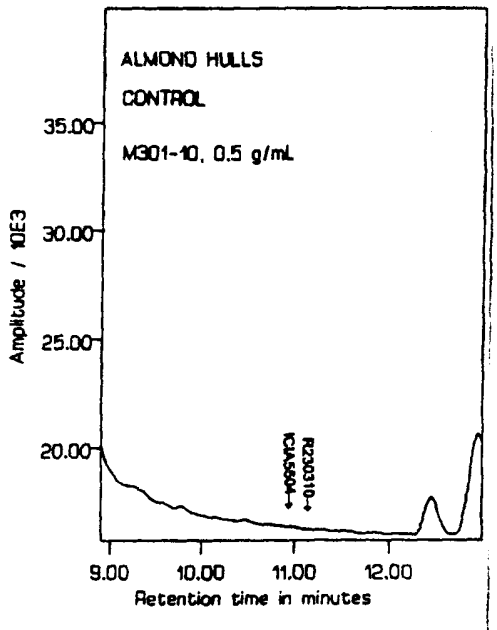
Typical Gas Chromatograms for Azoxystrobin and R230310 Determinations in Crops

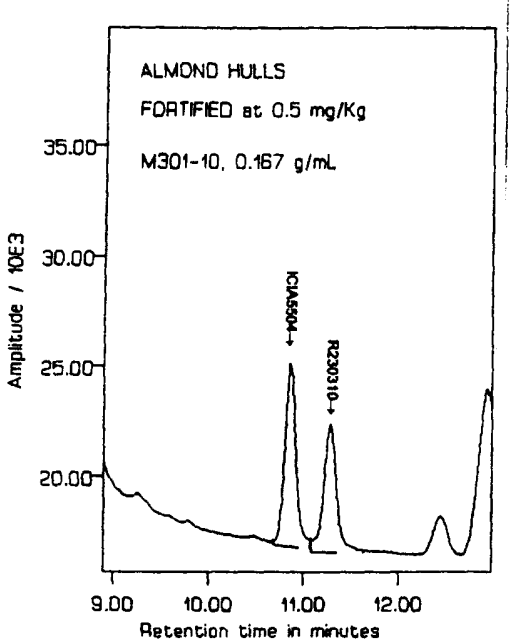
Figure 1. Representative Chromatograms for Almond Hull

Calibration Solution 0.1 µg/mL
ICIA5504 @ 0.1 µg/mL
R230310 @ 0.1 µg/mL

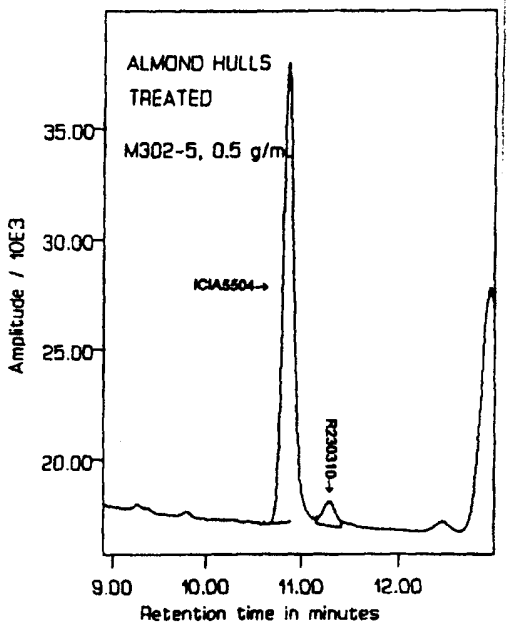


M301-10 Untreated Control: Hulls
C/Solv: 0.5 g/mL
ICIA5504: <0.01 mg/kg
R230310: <0.01 mg/kg





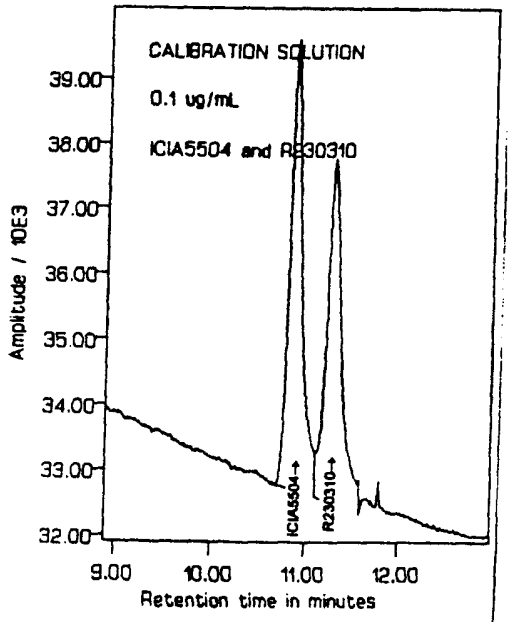
M301-10Fortified Recovery: 0.5 mg/kg
Crop to/Solvent: 0.167 g/mL
ICIA5504: 0.379 mg/kg, 76%
R230310: 0.351 mg/kg, 70%



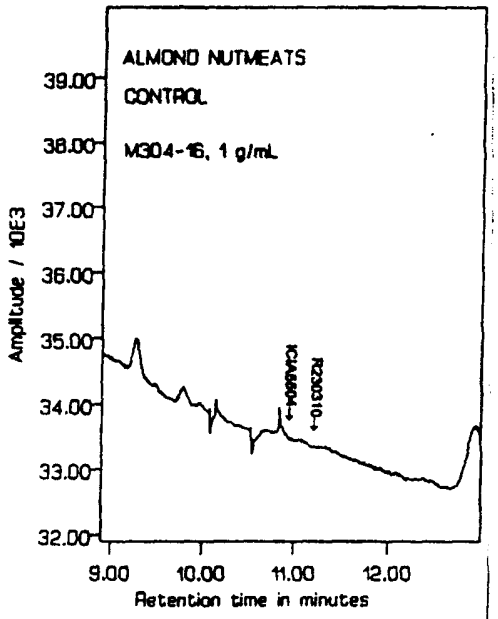
M302-5 Treated Hulls
Crop to/Solvent: 0.5 g/mL
ICIA5504: 0.93 mg/kg
R230310: 0.06 mg/kg

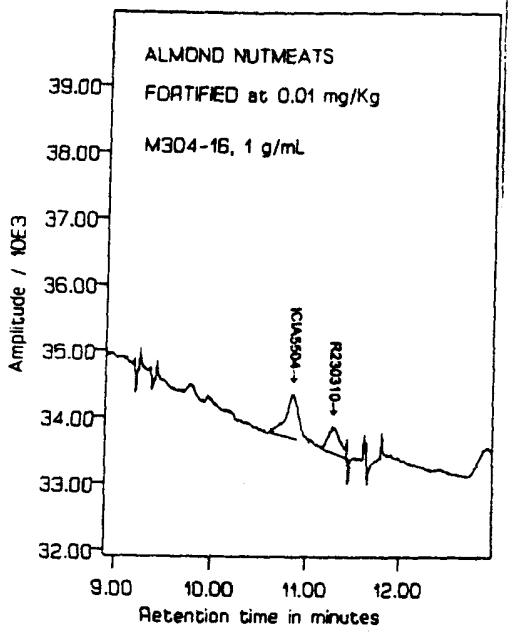
Figure 2. Representative Chromatograms for Almond Nutmeat

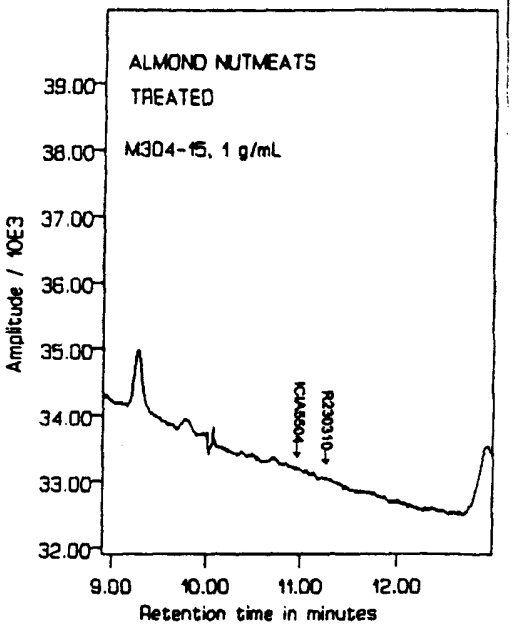
Calibration Solution 0.1 µg/mL
ICIA5504 @ 0.1 µg/mL
R230310 @ 0.1 µg/mL



M304-16 Untreated Control: Nutmeat
Crop/Solvent: 1.0 g/mL
ICIA5504: <0.01 mg/kg
R230310: <0.01 mg/kg



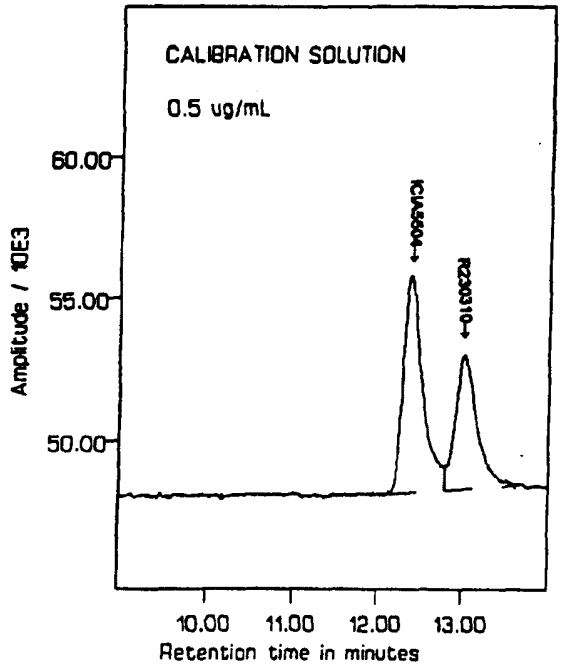




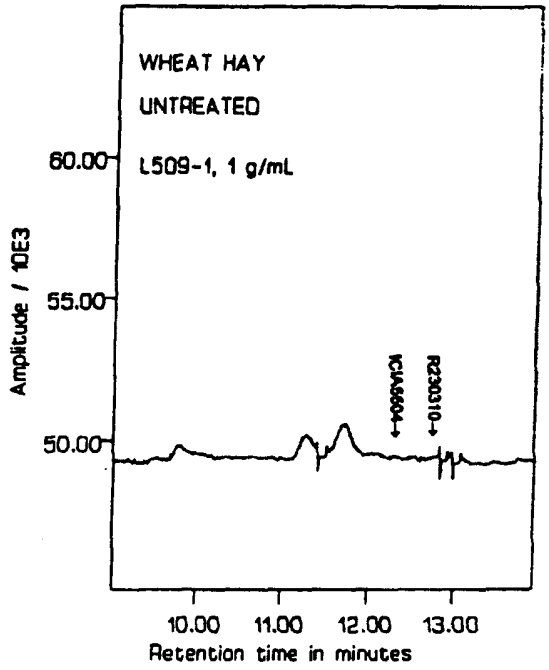
M304-16 Fortified Recovery: 0.01 mg/kg
Crop to/Solvent: 0.1 g/mL
ICIA5504: 0.009 mg/kg, 94%
R230310: 0.008 mg/kg, 75%

M304-15 Treated Nutmeat
Crop to/Solvent: 1.0 g/mL
ICIA5504: <0.01 mg/kg
R230310: <0.01 mg/kg

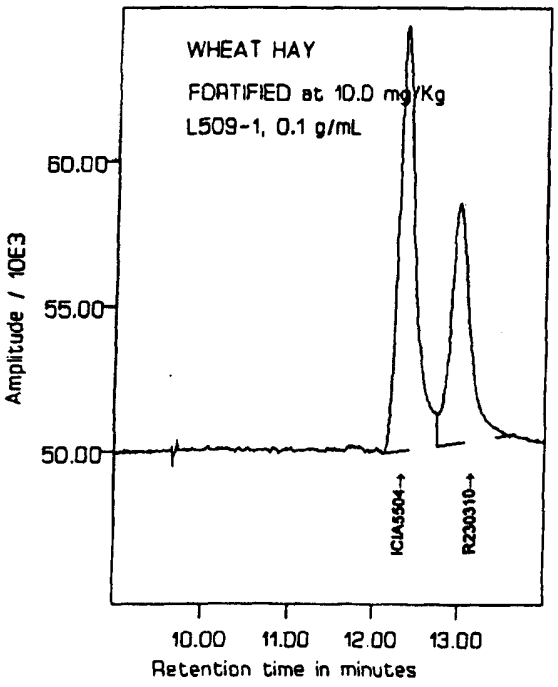
Figure 3. Representative Chromatograms for Wheat Hay



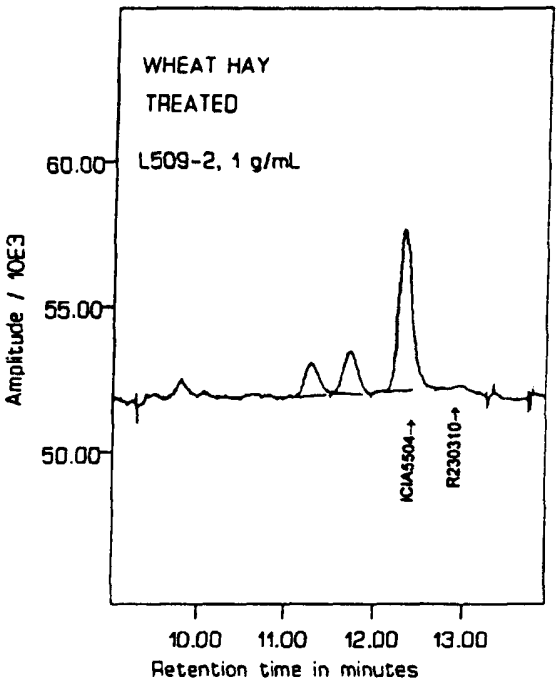
Calibration solution: 0.5 µg/mL
ICIA5504 ~ 12.5 minutes
R230310 ~ 13.1 minutes



L509-1 Wheat Hay, Untreated Check
sample/solvent = 1.0 g/mL
ICIA5504: <0.01 mg/kg
R230310: <0.01 mg/kg

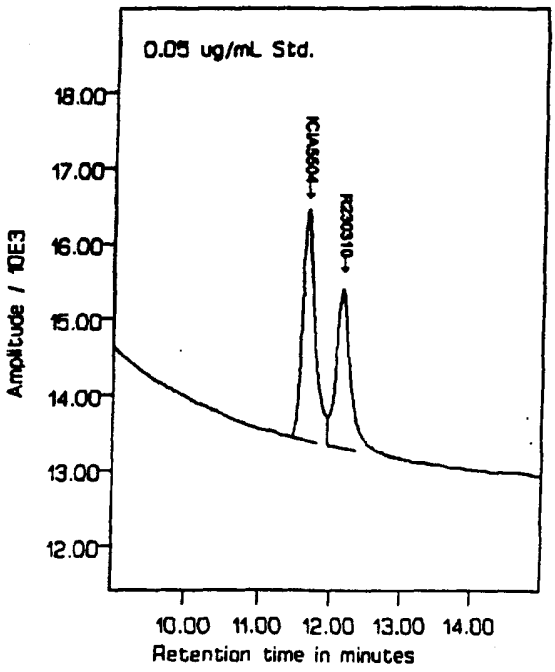


L509-1 Fortified @ 10.0 mg/kg
sample/solvent = 0.1 g/mL
Recovery ICIA5504: 9.22,mg/kg: 92%
Recovery R230310: 8.34 mg/kg: 83%

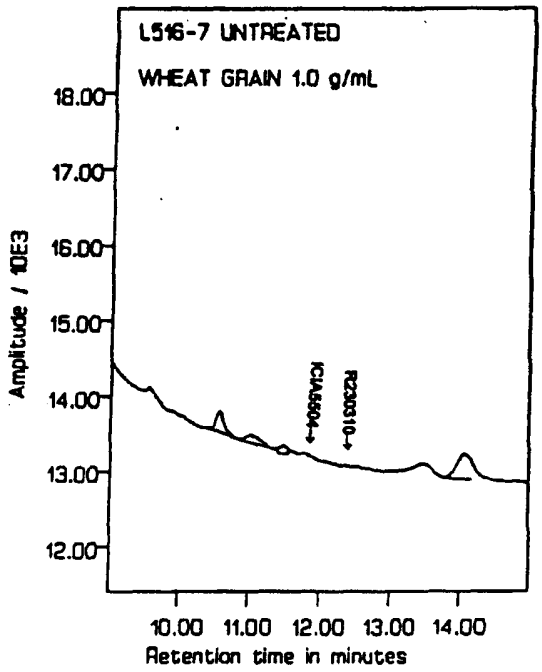


L509-2 Treated Wheat Hay
sample/solvent = 1.0 g/mL
ICIA5504: 0.32 mg/kg
R230310: 0.03 mg/kg

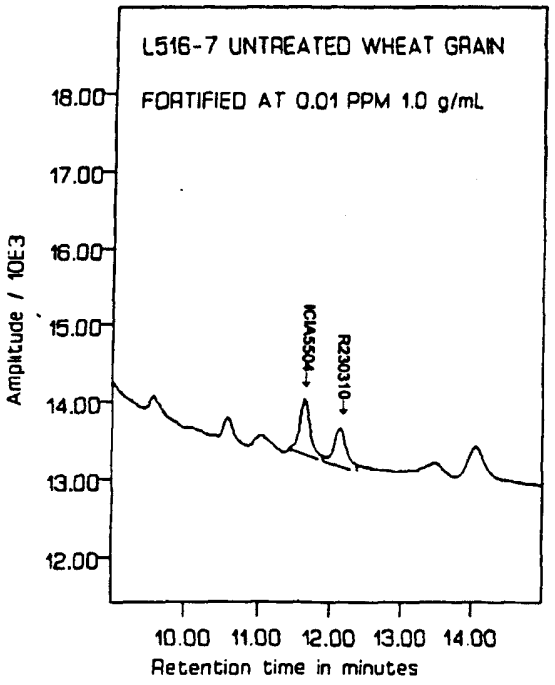
Figure 4. Representative Chromatograms for Wheat Grain



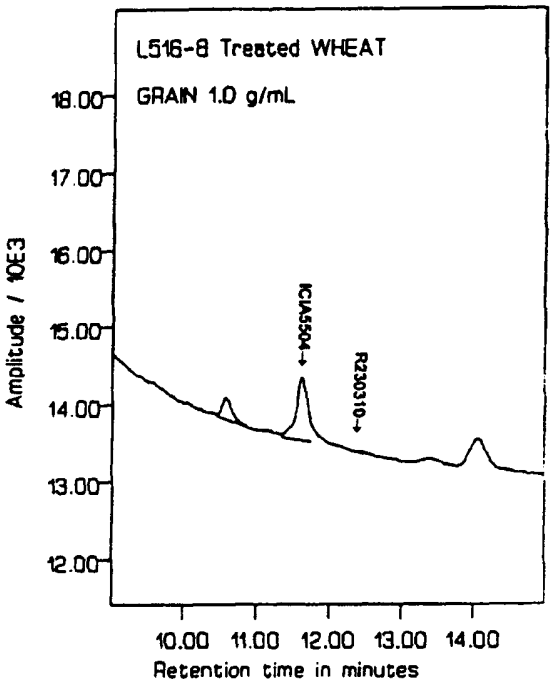
Calibration solution: 0.5 µg/mL
ICIA5504 ~ 11.7 minutes
R230310 ~ 12.3 minutes



L516-7, Wheat Grain, Untreated Check
sample/solvent = 1.0 g/mL
R230310: <0.01 mg/kg
ICIA5504: <0.01 mg/kg

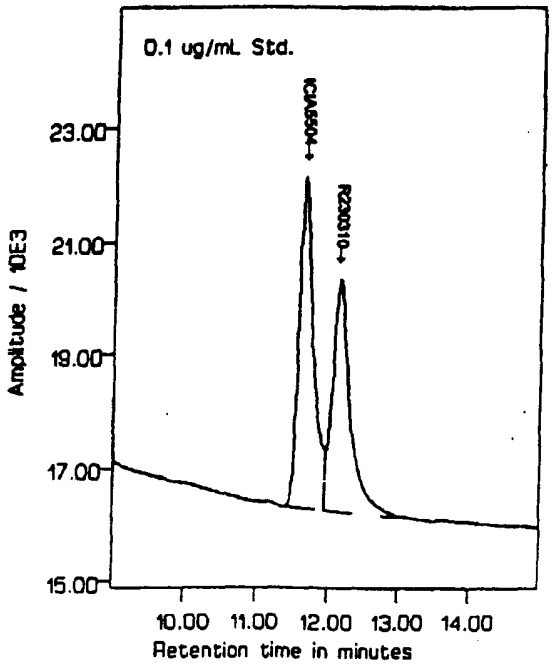


L516-7 Fortified @ 0.01 mg/kg
sample/solvent sd= 1.0 g/mL
Recovery ICIA5504: 0.011 ,g/kg: 113%
Recovery R230310: 0.011 mg/kg: 112%

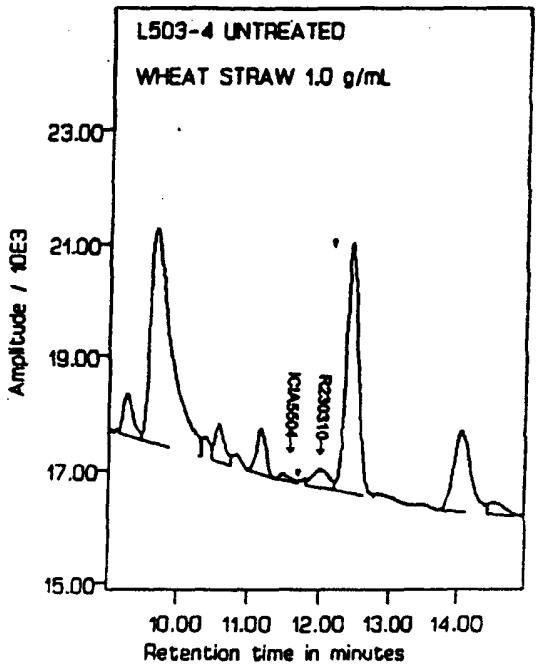


L516-8, Treated Wheat Grain
sample/solvent = 1.0 g/mL
ICIA5504: 0.01 mg/kg
R230310: <0.1 mg/kg

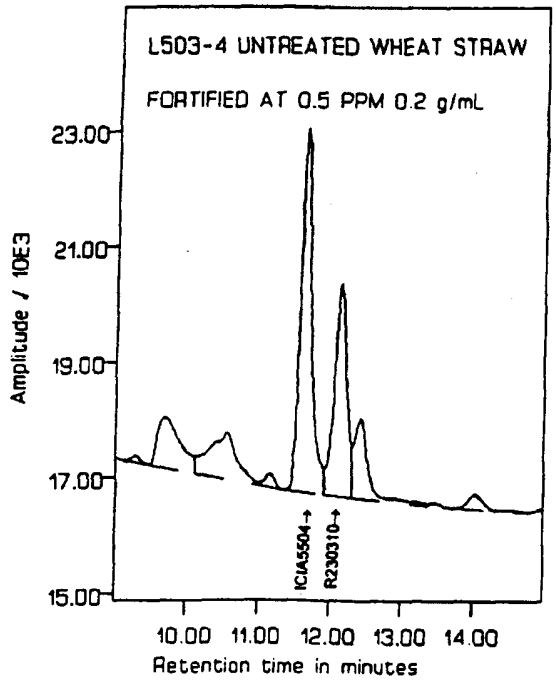
Figure 5. Representative Chromatograms for Wheat Straw



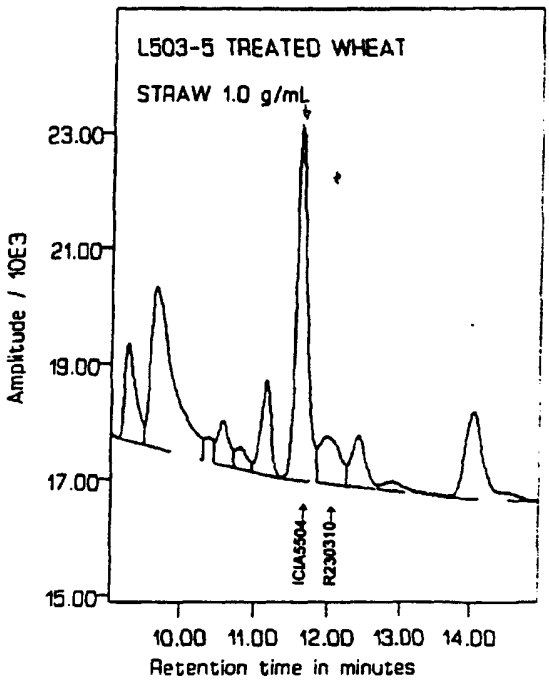
Calibration solution: 0.1 µg/mL
ICIA5504 ~ 11.7 minutes
R230310 ~ 12.3



L503-4, Wheat Straw, Untreated Check
sample/solvent = 1.0 g/mL
ICIA5504: <0.01mg/kg
R230310: <0.01 mg/kg



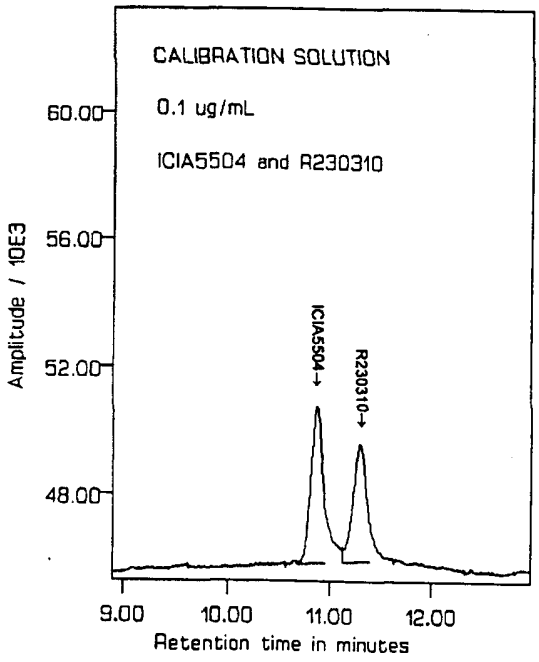
L503-4 Fortified @ 0.5 mg/kg
sample/solvent = 0.1 g/mL
Recovery ICIA5504: 0.503,mg/kg: 101%
Recovery R230310: 0.417 mg/kg: 83%



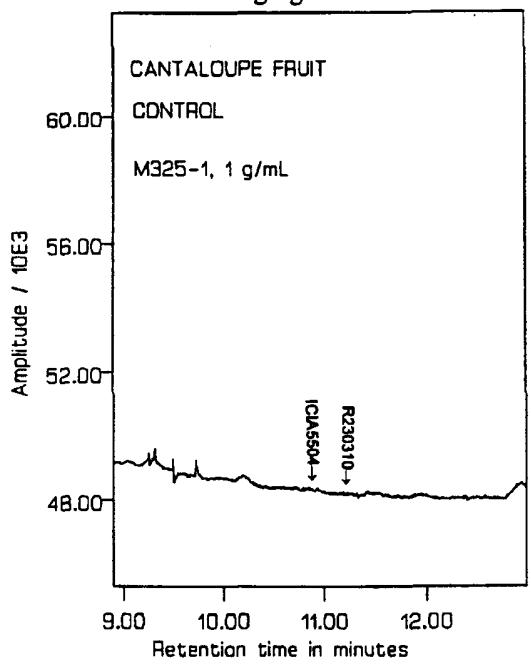
L503-5 Treated Wheat Straw
sample/solvent = 1.0 g/mL
ICIA5504: 0.10 mg/kg
R230310: <0.01 mg/kg

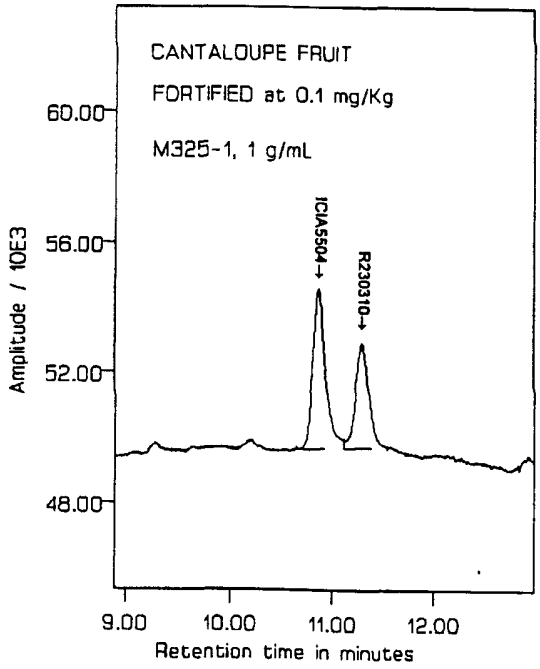
Figure 6. Representative Chromatograms for Cantaloupe

Calibration Solution 0.1 µg/mL
ICIA5504 @ 0.1 µg/mL
R230310 @ 0.1 µg/mL

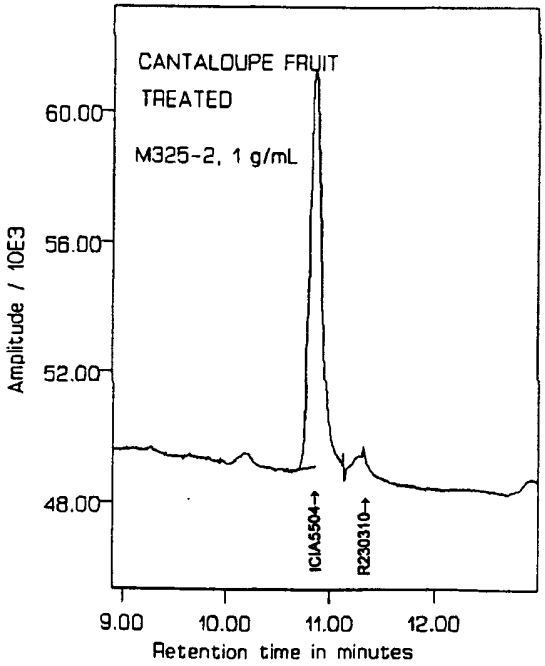


M325-1 Untreated Control: Cantaloupe
Crop/Solvent: 1.0 g/mL
ICIA5504: <0.01 mg/kg
R230310: <0.01 mg/kg





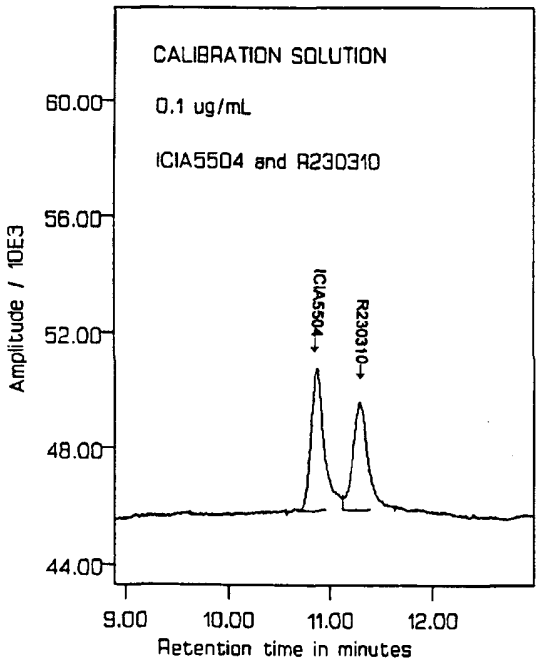
M325-1Fortified Recovery: 0.1 mg/kg
Crop to/Solvent: 1.0 g/mL
ICIA5504: 0.097 mg/kg, 97%
R230310: 0.085 mg/kg, 85%



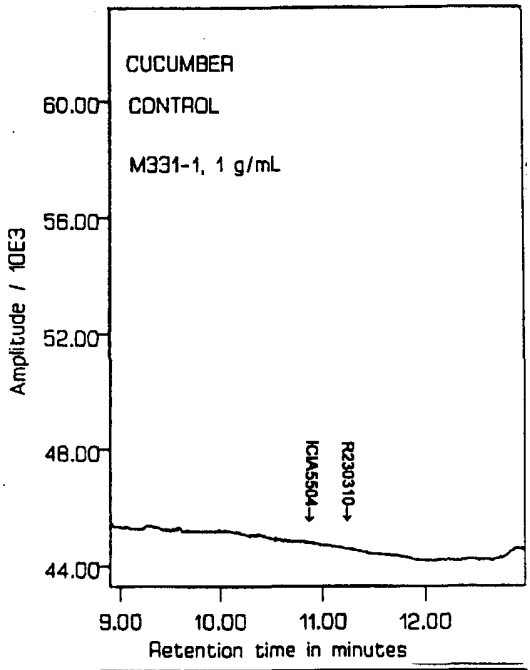
M325-2 Treated Cantaloupe
Crop to/Solvent: 1.0 g/mL
ICIA5504: 0.23 mg/kg
R230310: <0.01 mg/kg

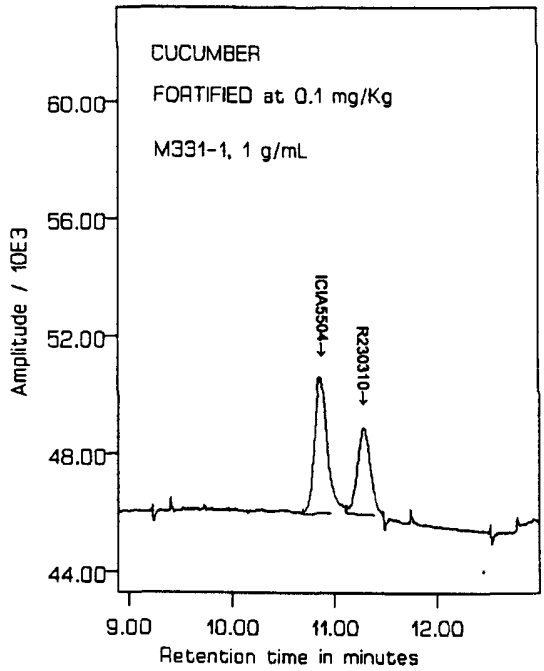
Figure 7. Representative Chromatograms for Cucumber

Calibration Solution 0.1 µg/mL
ICIA5504 @ 0.1 µg/mL
R230310 @ 0.1 µg/mL

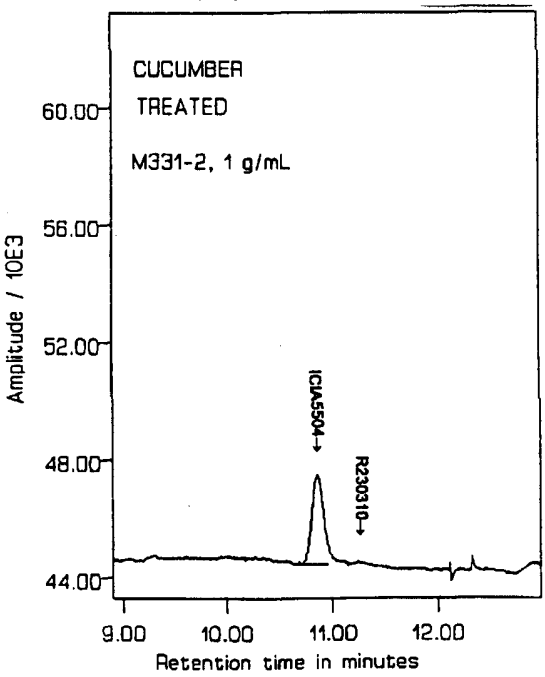


M331-1 Untreated Control: Cucumber
C/Solv: 1.0 g/mL
ICIA5504: <0.01 mg/kg
R230310: <0.01 mg/kg





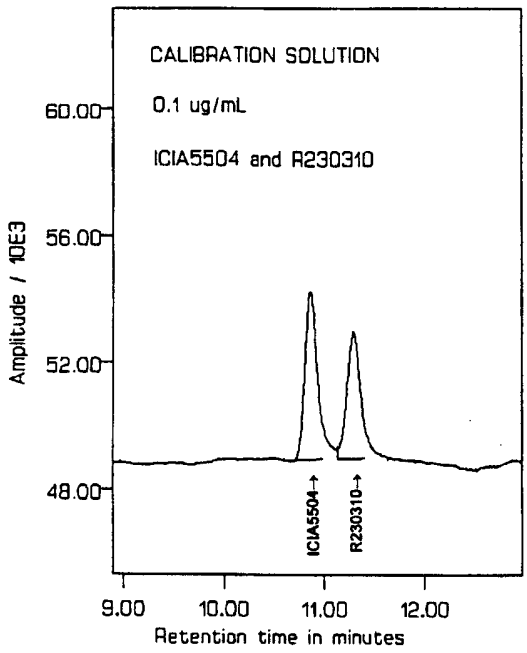
M331-1Fortified Recovery: 0.1 mg/kg
Crop to/Solvent: 1.0 g/mL
ICIA5504: 0.094 mg/kg, 94%
R230310: 0.089 mg/kg, 89%



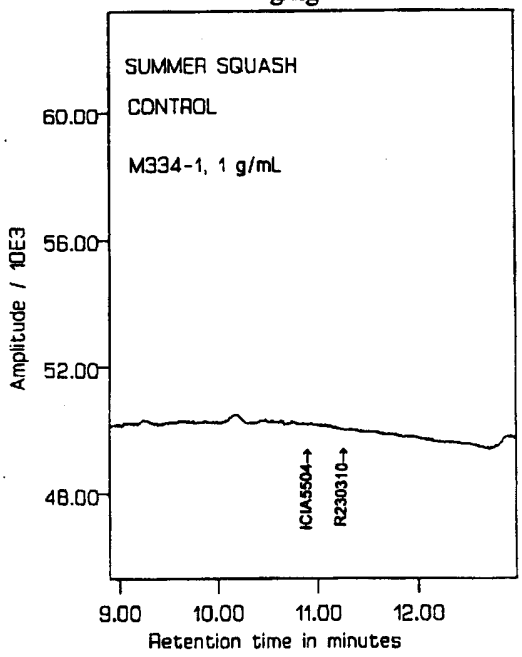
M331-2 Treated Cucumber
Crop to/Solvent: 1.0 g/mL
ICIA5504: 0.06 mg/kg
R230310: <0.01 mg/kg

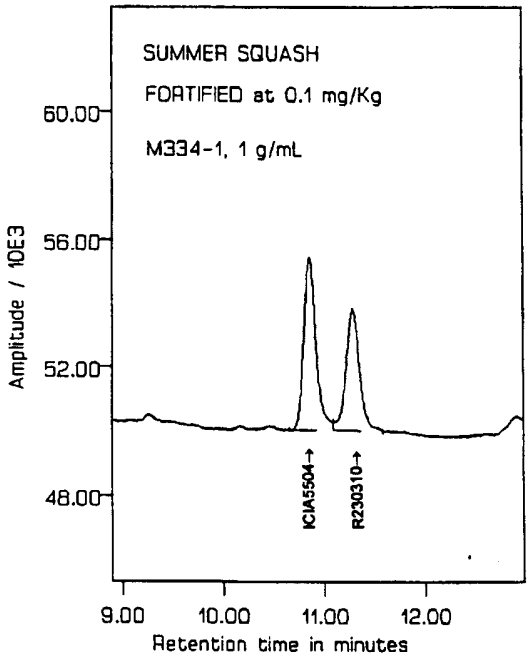
Figure 8. Representative Chromatograms for Summer Squash

Calibration Solution 0.1 µg/mL
ICIA5504 @ 0.1 µg/mL
R230310 @ 0.1 µg/mL

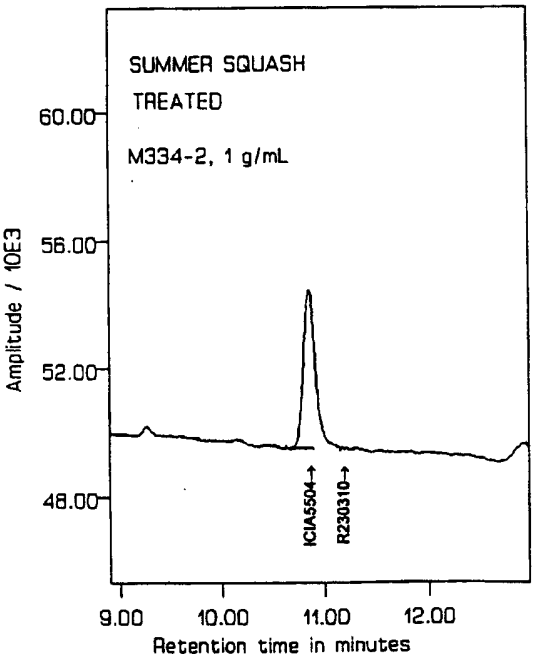


M334-1 Untreated Control: Summer Squash
Crop/Solvent: 1.0 g/mL
ICIA5504: <0.01 mg/kg
R230310: <0.01 mg/kg





M334-1Fortified Recovery: 0.1 mg/kg
Crop to/Solvent: 1.0 g/mL
ICIA5504: 0.102 mg/kg, 102%
R230310: 0.094 mg/kg, 94%



M334-2 Treated Summer Squash
Crop/Solvent: 1.0 g/mL
ICIA5504: 0.09 mg/kg
R230310: <0.01 mg/kg

Appendix 2
Materials / Safety

1 Apparatus

1. Gas Chromatograph: Hewlett-Packard (HP) 5890 with nitrogen-phosphorus detector (NPD) and temperature programmable injector
2. Analytical Column: Restek RTX-200, 30 m x 0.53 mm x 1 µm film thickness
3. Syringes for sample fortifications: 10, 25, and 250 µL capacity (Hamilton gas-tight 1700 series)
4. Analytical Balances: Mettler PM 2000 or Mettler PC 4400 for weighing of samples and Mettler PC440 for preparing stock solutions or equivalent
5. Glass Pipettes: 1, 2, 5, 10, and 25 mL disposable, graduated glass pipettes for general use
6. Graduated Cylinders: 10, 25, 50, 100 and 250 mL graduated glass cylinders
7. Separatory Funnels: 250 mL partitioning funnels with Teflon stopcocks
8. Rotary Vacuum Evaporator used for evaporating samples in boiling flasks
9. Volumetric Flasks: 10, 25, 50 and 100 mL capacities
10. Pasteur disposable glass transfer pipettes
11. Omni-mixer, suitable for use with pint mason jars or blender
12. Pint Mason jars or blender jars
13. Whatman # 1 filter paper, 9 cm diameter circles
14. Buchner funnels, 100 mm diameter
15. Filter flasks or vacuum flask adapters to fit 24/40 glass joint on boiling flasks
16. Flat or round-bottom boiling flasks, 100 and 250 mL capacity
17. Glass filtering funnels, 50 mm diameter
18. Glass wool
19. Florisil columns (Fisher Prepsep P466R)
20. Glass chromatographic column, 10 mm diameter
21. Autosampler vials for GC analysis

2 Reagents

1. Extraction Solvent: acetonitrile:water 90:10 (v/v)
2. Water: deionized water or distilled water
3. Solvents: acetonitrile, ethyl acetate, acetone, methanol, hexane, toluene, dichloromethane: Fisher Optima grade or equivalent
4. Sodium sulfate: granular and anhydrous
5. Analytical Reference Standard of azoxystrobin and R230310 of known purity. Available from Zeneca Ag Products, 1200 South 47th Street Richmond, CA 94804-0023
6. Sodium chloride 5% (w/v) solution
7. Florisil (100-200 mesh)

3 Safety Precautions

Personnel untrained in Good Laboratory Practices and the routine safe handling of chemicals must not attempt to use this procedure. Information on hazards and first-aid procedures can be found in the Material Safety Data Sheets accompanying the chemicals, or are available from the chemical supplier. In general, always wear safety glasses with side shields, work in a well-ventilated area, avoid inhaling vapors, and avoid contact of the chemicals with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

4 Analytical Reference Standards

Separate stock fortification solutions of azoxystrobin and R230310, 1000 µg/mL, were prepared by weighing 50 ± 1 mg of the reference standard into a 50 mL volumetric flask and diluting with acetone to the calibration mark. Working fortification solutions combining both analytes were prepared by serial dilution with acetone.

Separate stock calibration solutions of azoxystrobin and R230310, 1000 µg/mL, were prepared by weighing 50 ± 1 mg of the reference standard into a 50 mL volumetric flask and diluting with toluene to the calibration mark. Working calibration solutions combining both analytes were prepared by serial dilution with toluene. Stock solutions may be stored in the refrigerator for one year at 7°C.