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STANDARD OPERATING PROCEDURE

RAM 305/03

RESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF AZOXYSTROBIN (ICI5504) AND R230310 IN CROP SAMPLES. FINAL DETERMINATION BY LC-MS/MS.

Effective Date	: 25 November 2004	**Review Date**	: Annually
Author	: S Chaggar, S J Crook, E A Harron and N J Robinson	**Issuing Section**	: Dietary Safety
Reviewer	: n/a		
Authorised by	: _____	**T Clark** Group Leader	7/11/04 Date

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Summary of revisions to previous version

Version	Summary of Revisions
03	Simplified procedure with 0.01g of matrix extracted.
	Primary and confirmatory ions monitored for each analyte.
	Replacement of acetonitrile in the mobile phase with methanol if interfering peaks observed.
	Applied Biosystems API 3000 triple quadrupole MS used for all analyses.
	Silica solid phase extraction clean up removed.

Contents

1. Introduction and Summary

1.1 Scope

The analytical procedure described is suitable for the determination of residues of azoxystrobin and R230310 (Figure 1 and 2) in crop samples using an external standardisation procedure. The limit of quantification has been set at 0.01 mg kg^{-1}.

Figure 1 : Azoxystrobin

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

Molecular Mass : 403.4

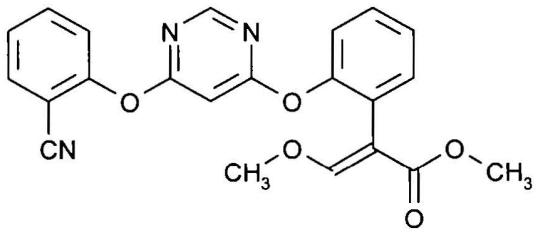
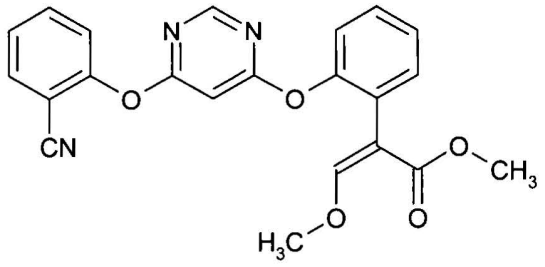


Figure 2 : R230310

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

Molecular Mass : 403.4



1.2 Method Summary

Samples are extracted by homogenisation with acetonitrile:water. After centrifugation, an aliquot is removed and cleaned up using a C_{18} solid phase extraction procedure. Final determination is by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer in selected reaction monitoring mode (LC-MS/MS).

2. Materials

The recommended equipment and reagents are described in Appendices 1 and 2. Equipment with equivalent performance specifications and reagents of comparable purity can be substituted provided that they can be shown to be suitable.

2.1 Apparatus

See Appendix 1 for a list of apparatus used during this method.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled or HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. See Appendix 2 for a list of reagents used in this method.

2.3 Preparation of Analytical Standards

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

Weigh out accurately, using a five-figure balance, sufficient azoxystrobin or R230310 analytical standard to allow dilution in 50:50 (v/v) acetonitrile:water , to give a 200 $\mu g\ mL^{-1}$ stock solution in a volumetric flask. This stock solution should then be diluted by serial dilution to prepare mixed or individual azoxystrobin and R230310 standards in 50:50 (v/v).acetonitrile:water. These

should be used as fortification and calibration standards for final determination by LC-MS/MS.

When not in use, always store the standard solutions in a refrigerator at ≤7°C to prevent decomposition and/or concentration of the standard. Unless and until further stability information is obtained, analytical standards should be replaced with freshly prepared standards after four months.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate safety manual (e.g. Syngenta Laboratory Safety Manual), which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by S G Luxon, The Chemical Society, London (Reference 1).

Solvent Hazards

	Acetonitrile	Methanol	Dichloromethane	Ethyl acetate	Hexane
Harmful Vapour	✓	✓	✓	✓	✓
Highly flammable	✓	✓	×	✓	✓
Harmful by skin absorption	✓	×	✓	✓	✓
Syngenta Hazard Category (SCH)	C	C	D	B	B
OES short term (mg m^{-3})	105	310	870 (MEL)	-	3600
OES long term (mg m^{-3})	70	260	350 (MEL)	1400	70

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

Azoxystrobin has been assigned the Syngenta Hazard Category C.

At present there is insufficient data available to assign a Syngenta Hazard Category for R230310, it should be treated as a SCH-C compound until further information indicates otherwise.

The hazard category scale rates highly hazardous substances as category E and non-hazardous substances as category A.

2.5 Time Required for Analysis

The methodology is normally performed with a batch of up to 20 samples. One person can complete the analysis of up to 20 samples in 1 day (8 working hour period).

2.6 Work Stoppages

The analytical procedure can be stopped at various points for overnight and weekend breaks except where specified in the analytical procedure. Acceptable external standard recoveries will validate the work stoppages. Samples should be stored in sealed vessels at a temperature of ≤7°C.

2.7 Modifications and Potential Problems

a) For preparation of aqueous HPLC mobile phases it has been found beneficial to use bottled HPLC grade water. This gives a reduced MS/MS background signal when compared to water from a laboratory water purification system.

b) If interfering peaks effecting resolution are observed (e.g. mandarin) then it is recommended that the acetonitrile in the mobile phase be replaced by methanol.

3. Analytical Procedure

3.1 Sample Preparation

Samples should be prepared using an approved method of sample preparation for residue analysis, such as Syngenta standard operating procedure ESJH/910/-- for crops (Reference 2).

3.2 Extraction.

a) Weigh representative amounts of crop (10 g) or straw (5 g) into polypropylene centrifuge bottles (250 mL size). At least one untreated control and two control samples fortified with known amounts of each analyte in 50:50 acetonitrile:ultra pure water should be analysed alongside each batch of samples to demonstrate acceptable method performance.

Add acetonitrile: UP water (90:10 v/v;100 mL minus the water content of the samples) (Reference 4 - 6). Homogenise at high speed for 3 – 5 minutes.

Note: Estimate the percentage water content in each matrix type and hence the total volume of water in the 10 g sub-sample. E.g. for a 10 g sub-sample with 90% natural water content add 100 mL – (10 x 90/100) mL = 91 mL extraction solution. It is sufficient to round the natural water content to the nearest ten

percent value. Any volume contraction due to mixing organic solvents with water and evaporation loss during extraction is considered to be negligible.

The water content of matrices can be obtained from published sources (e.g. Reference 6). The relevant information may also be obtained from the following USDA web site: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl

Alternatively, where information is not available from the such sources, it may be necessary to determine the moisture content experimentally, following a suitable moisture content determination procedure e.g. SOP ESJH/309/-- (Reference 7).

b) Centrifuge samples at 3500 rpm (or at a speed that visibly separates the solid sample from the supernatant) for 5 minutes.

The sample concentration is now 0.1 g mL^{-1} or 0.05 g mL^{-1} for straw.

3.3 Sample Dilution

a) Transfer aliquots of the crop extracts equivalent to 0.01 g (0.1 mL) or for straw extracts (0.2 mL) into appropriate vessels (e.g. test tube).

b) Add acetonitrile:ultra pure water (30:70 v/v) to give a final volume of 5 mL and mix well.

3.4 Solid Phase Extraction

a) Take one Isolute C_{18} (EC) (6 mL, 1 g size) solid phase extraction cartridge for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add methanol (5 mL) and draw through under vacuum to the level of the top frit at a rate of approximately 2 mL min^{-1}, discarding the column eluate. Add 30:70 (v/v) acetonitrile:water (5 mL) to the top of each column and draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow columns to become dry.

b) Transfer the sample aliquots from section 3.3 (b) onto the columns and allow to percolate through under gravity or low vacuum (approx. 200 mbar), discarding the column eluates. Azoxystrobin and R230310 will now be retained on the column.

c) Rinse out the flasks that contained the samples with 30:70 (v/v) acetonitrile: up water (5 mL), add to the top of each column and draw through under vacuum to the level of the top frit, at an approximate speed of 2 mL min^{-1}. Discard column

eluates. Remove any remaining droplets of water adhering to the inside of the column with absorbent tissue.

d) Dry the columns under high vacuum (≤500 mbar) for a minimum of 15 minutes.

Note : Where achievable vacuums are less than specified or apparatus does not allow sufficient air flow through the columns, longer drying times may be required.

e) Add hexane (5 mL) to the top of each column and draw through under vacuum to the level of the top frit at an approximate speed of 2 mL min^{-1}, discarding the column eluates.

f) Place a rack containing collection test tubes (10 mL size) under each port, as required, in the manifold. Add 55:45 (v/v) ethyl acetate:dichloromethane solution (5 mL) to the top of the columns and draw through the cartridge under vacuum to the level of the top frit, at an approximate speed of 2 mL min^{-1}. The azoxystrobin and R230310 are eluted in this fraction.

Note: The above SPE procedure has been developed using columns from the stated manufacturer, however, it is possible to carry out the procedure using similar columns from other manufacturers. In all cases, it is strongly recommended that the elution profile of the chosen batch of columns is checked prior to commencing analysis to rule out any variation between manufacturers' products and between batches.

g) Evaporate the samples to dryness under a stream of clean, dry air. Elevated temperatures of up to 50°C may be used to aid this process.

h) Dissolve the samples in 50:50 acetonitrile: UP water (v/v) (1 mL). Ultrasonicate thoroughly to ensure complete uptake and transfer to autosampler vials ready for LC-MS/MS analysis. If samples are visibly cloudy or contain particulate material filter through Gelman GHP Acrodisc 0.45 μm syringe filters, before transferring to autosampler vials. The final sample concentration is 0.01 g mL^{-1} for all crops.

3.5 LC-MS/MS Calibration Standards

Calibration standards for LC-MS/MS analysis are prepared as described in Section 2.3.

Suppression or enhancement of LC-MS/MS response to azoxystrobin and R230310 in the presence of matrix was generally less than 10% in this laboratory on the crop matrices tested. This is considered to be negligible and samples should be quantified using non-matrix matched standards.

If greater suppression or enhancement is observed, a matrix-matched standard may be used to compensate at the discretion of the study director. For example, to prepare a 0.001 μg mL^{-1} azoxystrobin matrix matched standard, take a further 0.1 mL control aliquot at section 3.3 (a). Take the sample through the analytical procedure to section 3.4 (h). Add 1.0 mL of a 0.001 μg mL^{-1} standard in acetonitrile:ultra pure water (50:50, v/v) to the evaporated sample. Ultrasonicate thoroughly and transfer to a suitable autosampler vial. The concentration of the matrix matched calibration standard is 0.001 μg mL^{-1}.

4. Final Determination by LC-MS/MS

The following instruments and conditions have been found to be suitable for this analysis in this laboratory. Other instruments can be equally used, however optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

4.1 Instrument Description

Pump	:	Agilent 1100 series quaternary pump model number G1311A
Degasser	:	Agilent 1000 series model number G1322A
Column Oven	:	Agilent 1100 series model number G1316A
Detector	:	Applied Biosystems API 3000 triple quadrupole mass spectrometer with Analyst™ software version 1.3.1
Auto sampler	:	CTCPAL
Gas Supply	:	Peak Scientific NM20ZA gas station

4.2 Chromatography Conditions

Column	:	KR100 5C18 5μm 50 mm x 3.2 mm i.d
Column Oven Temperature	:	40°C
Flow rate	:	1.0 mL min^{-1}

Injection volume	:	20 - 30 µL
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Stop Time	:	2 minutes (or 5.5 minutes if using methanol)
Mobile phase	:	**1** : Acetonitrile (or Methanol) **2** : 0.2% (v/v) acetic acid in UP water

Mobile Phase Gradient

Time (min)	% 1	% 2
0.0	50.0	50.0
2.0 (or 5.5)	50.0	50.0

Column Divert programme

Time (min)	Valve position
0.0	To waste
0.75	To mass spectrometer

Note : The column eluate is diverted to waste for 0.75 minutes to prevent ionic material from the sample contaminating the mass spectrometer front plate. A secondary pump providing a flow of mobile phase to the mass spectrometer when the column eluate is switched to waste is unnecessary.

The flow of eluate into the mass spectrometer should be optimised using an in-line flow splitter. A flow rate of approximately 200 – 400 $\mu L\ min^{-1}$ is recommended. This should produce a small wet spot visible on the MS front plate at the start of the injection.

Under these conditions the retention time of azoxystrobin and R230310 is approximately 1.22 and 0.97 minutes respectively when acetonitrile is used for the mobile phase. When methanol is used for the mobile phase the retention times of azoxystrobin and R230310 are 3.83 and 2.70 minutes respectively.

4.3 API 3000 Mass Spectrometer Conditions

Interface	:	TurboIonSpray
Polarity	:	Positive
Nebuliser gas (NEB)	:	Air set at 13 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 12 (arbitrary units)
Temperature (TEM)	:	450°C
Ionspray voltage	:	5000 V
Collision gas setting (CAD)	:	Nitrogen set at 4 (arbitrary units)
Scan type	:	MRM
Q1 *m/z*	:	404.2
Q3 *m/z*	:	372.4 & 343.8
Dwell time	:	400 ms (or 700 ms if using methanol)
Resolution Q1	:	Low
Resolution Q3	:	High

		Primary transition	Confirmatory transition
Declustering potential (DP)	:	41.0 V	36.0 V
Focusing potential (FP)	:	260.0 V	270.0 V
Entrance potential (EP)	:	10.0 V	10.0 V
Collision energy (CE)	:	21.0 V	35.0 V
Collision cell exit potential (CXP)	:	24.0 V	10.0 V
Electron multiplier setting (CEM)	:	1800 V	1800 V

Protonated molecular ions generated in the ion source (*m/z* 404.2) are selected and subjected to further fragmentation by collisional activation. The most abundant two ions (*m/z* 372.4, corresponding to loss of methanol and *m/z* 343.8, corresponding to the loss of a methoxy group) in the resulting daughter spectra are then monitored. LC-MS/MS is considered to be highly specific therefore only the *m/z* 404.2 → 372.4 transition is used for quantitative analysis, however a second transition (*m/z* 404.2 → 343.8) may also be monitored if further confirmation is required. Typical chromatograms are shown in Appendix 4. Initial and final product scans showing the fragmentation and daughter ions for azoxystrobin and R230310 are presented in Appendix 6.

5. Calculation of Results

Residues may be calculated using an external standardisation procedure.

Azoxystrobin and R230310 residues may be calculated in mg kg^{-1} for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing azoxystrobin and R230310 at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak area obtained for azoxystrobin and R230310.

b) Make an injection of each sample solution and measure the peak heights or areas of the peaks corresponding to azoxystrobin and R230310.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the azoxystrobin and R230310 residue in the sample, expressed as mg kg^{-1}, using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample

PK area (STD) = Average peak response for bracketing standards

Standard Conc. = Concentration of calibration standard (μg mL^{-1})

Sample Conc. = Sample concentration (g mL^{-1})

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average Percentage Recovery}} \ (\text{mg kg}^{-1})$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

6. Control and Recovery Experiments

Fortification levels for procedural recoveries should be appropriate to the residue levels expected. A minimum of one control and two external recovery experiments should be run alongside each set of samples analysed (that is untreated samples accurately fortified with a known amount of azoxystrobin and R23310 prior to extraction).

Control and external recovery experiments should be completed as section 3 for each set of samples analysed. Acceptable recovery values demonstrate reliable method performance during the analysis and may be used to correct any azoxystrobin and R23310 residues found.

Recovery data is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation (RSD) of $\leq$20%.

7. Specificity

If unexpected interference is observed at final determination, it is recommended that a reagent blank is taken through the analytical procedure to trace the source of the problem. Reagent blanks may be taken through the analytical procedure on a routine basis.

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Interference arising from the crop matrices tested has not been observed except for mandarins. For this matrix the acetonitrile contained in the mobile phase should be replaced by methanol to achieve acceptable resolution between the analytes and interfering peaks.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents, no reagent interference has been found.

7.3 Labware Interference

The method mainly uses disposable labware. No interference from labware has been found.

7.4 Protocol for High Level Standard and Sample Residue Injection

It is recommended when analysing standards and sample residues at high concentration (e.g. ≥0.01 $\mu g\ mL^{-1}$) that carry-over effects into subsequent injections are checked. Blank samples containing mobile phase may be injected after high concentration samples and standards to prevent carry over.

8. Method Validation

8.1 Recovery Data and Repeatability

A method validation study demonstrating acceptable recovery data and repeatability has been carried out on the procedures described in Section 3 and 4. This is reported in RJ3552B (Reference 8). A summary of the method validation data is presented in Appendix 3.

8.2 Limit of Quantification and Limit of Detection

8.2.1 Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70 - 110% with a % RSD of ≤ 20% has been obtained.

Generally, for accurate quantification, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantification has been set at 0.01 $mg\ kg^{-1}$ for LC-MS/MS determination.

8.2.2 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as four times background noise. Note that the LOD may vary between runs and from instrument to instrument.

The limit of detection of this method was estimated at 0.005 mg kg^{-1} for both the primary transition *m/z* 404.2 → 372.4 and confirmatory transition *m/z* 404.2 → 343.8

8.3 Detector Linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of detector responses. Detector linearity graphs are given in Appendix 5.

In these laboratories the linearity of the API3000 HPLC-MS/MS detector response for azoxystrobin and R230310 standards prepared in acetonitrile:ultra pure water (50:50, v/v) was tested in the range from 0.00005 to 0.2 μg mL^{-1} concentration (equivalent to 0.001 - 4 ng injected on column when using a 20 μL injection volume and equivalent to 0.0015 - 6 ng when using a 30 μL injection volume) and was found to be linear for both primary and confirmatory transitions. This was carried out using the mobile phase, acetonitrile:0.2% (v/v) acetic acid in UP water (50:50) (20 μL injection volume) and also repeated using methanol:0.2% (v/v) acetic acid in UP water (50:50) (30 μL injection volume) as the mobile phase.

Standards were injected in triplicate and the mean response plotted against amount injected, using Microsoft Excel 2000. The intercept was set to zero and a linear trendline fit applied. The data were also plotted with no intercept set. The two plots were compared statistically by application of a t-test, performed using the Simple Linear Regression Programme Version 2.0. The detector linearity graphs with the intercept set at zero were similar to the graphs with no intercept set. The linearity graphs with no intercept set are presented in Appendix 5.

T-Test Values

Transition tested	Concentration range ($\mu g\ mL^{-1}$)	Degrees of freedom (n-2)*	T-test value		Theoretical t-test value at 5 %
			Mobile phase acetonitrile: 0.2% acetic acid in water	Mobile phase methanol: 0.2% acetic acid in water	
Azoxystrobin (*m/z* 404.2 → 372.4)	0.00005 - 0.2	3	0.875	0.869	3.182
Azoxystrobin (*m/z* 404.2 → 343.8)	0.00005 - 0.2	3	0.845	0.863	3.182
R230310 (*m/z* 404.2 → 372.4)	0.00005 - 0.2	3	0.887	0.869	3.182
R230310 (*m/z* 404.2 → 343.8)	0.00005 - 0.2	3	0.885	0.887	3.182

*n= no of concentrations tested.

Since the computed t value is smaller than the tabular t value, at the 5% level of significance, the intercept α is not significantly different from zero and the two response curves are statistically similar. It is therefore acceptable to use single point calibrations for residue calculations (Reference 9).

If residues beyond the tested concentration range are expected, dilute the extract appropriately with 50:50 acetonitrile: up water (v/v) to bring it within the tested linear range prior to quantification.

8.4 Limitations

The method has been tested on representative crop types. It can be reasonably assumed that the method can be applied for other crop types not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

9. Conclusions

The method described is suitable for the analysis of azoxystrobin and R230310 residues in crops. Only commercially available laboratory equipment and reagents are required. One person can complete the analysis of a batch of up to 20 samples in 1 day (8 working hour period). Untreated and fortified samples should be extracted and analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification has been set at 0.01 mg kg^{-1} with final analysis by HPLC MS/MS.

10. References

1. Luxon S G (1992): Hazards in the Chemical Laboratory 5th Edition. The Royal Society of Chemistry. Thomas Graham House, The Science Park, Cambridge CB4 4WF, UK. ISBN 0-85186-229-2.

2. Syngenta Standard Operating Procedure SOP ESJH/910/--: Preparation of Crop Samples For Residue Analysis.

3. Lister N J (1999): Azoxystrobin : Validation of SOP RAM 305/01 for the Determination of Azoxystrobin and R230310 in Crops. ZENECA Agrochemicals Report Series RJ2770B.

4. Clarke D M, Sapiets A (1994) : ICIA5504 and R230310 : Validation of a method for the Determination of Residues in Cereals and Vines. ZENECA Agrochemicals Report Series RJ1557B.

5. Lister N, Hughes A (1999) : Azoxystrobin : Residue levels in Hops from Trials Carried Out in the UK During 1998 Report Series RJ2801B.

6. Watt B K and Merril A L (1975): Composition of Foods, raw, processed and prepared, Agricultural Handbook No.8, Agricultural Research Service, United States Department of Agriculture, US Government Printing Office, Washington, D C, 20402.

7. Syngenta Standard Operating Procedure SOP ESJH/309/--: Crop Moisture Determination.

8. Chaggar S (2004): Azoxystrobin (ICI5504) and R230310: Validation of Analytical Method RAM 305/03 for the Determination of Residues in Crops. Final Determination by LC-MS/MS.Syngenta Report Number RJ3552B.

9. Cardone M J, Palermo P J and Sybrandt L B : Potential error in single point ratio calculations based on linear calibration curves with a significant intercept. Anal Chem., 52 pp 1187-1191, 1980.

10. Burke S R : Azoxystrobin and R230310 Storage Stability in Acetonitrile:Water at Specified Temperatures for up to Six Months. Issue date Jan 2000. Report RJ2893B.

Filename : RAM305/03/--.doc
Location : E:/GROUP/WP/SOPRAM
Reference :
SYPOS
Task no. : T005372-04
Date : 12 November 2004

Appendices

Appendix 1 : Apparatus

UK Suppliers

Tecator homogeniser for initial preparation of samples available from Philip Harris Scientific, 618 Western Avenue, Park Royal, London W3 0TE, UK. Part number M48-525.

General laboratory glassware, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Plastic centrifuge bottles, 250 mL size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

High speed homogeniser for extraction of samples e.g. Janke and Kunkel Ultra Turrax T25, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number TWK-605-020C.

Laboratory centrifuge e.g. MSE Mistral 1000 series, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number CEK-151-010W

Disposable test tubes (10 mL capacity) available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number CEK-151-010W

Isolute Vacmaster-20™ sample processing station, available from Jones Chromatography Ltd., Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan CF8 8AU, UK.

Isolute C18 (EC) solid phase extraction columns, 6 mL 1 g size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number 221-0100-C.

Techne Dri-block 3D sample concentrator, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number BLD-750-010Y.

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number BMA-100-020P.

Crimp cap auto sampler vials and caps available from Agilent Technologies UK Limited, Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire. SK8 3GR.

API3000 HPLC-MS-MS system equipped with a TurboIonSpray source, available from Applied Biosystems, Kelvin Close, Birchwood Scientific Park North, Warrington, Cheshire WA3 7PB, UK.

HPLC column, KR100 5C18 50 mm x 3.2 mm i.d., 5 µm particle size, available from Hichrom Ltd, 1 The Markham Centre, Station Road, Theale, Reading Berkshire RG7 4PE, UK.

Agilent 1100 HPLC system equipped with a quaternary pump, vacuum degasser and column compartment with column switching valve, available from Agilent Technologies UK Limited, Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire SK8 3GR.

CTC HTS PAL auto sampler, available from Presearch Ltd, System House, 59-61 Knowlpiece, Hitchin, Herts SG4 0TY, UK.

Peak Scientific NM20ZA gas station, available from available from Peak Scientific Instruments Ltd, Fountain Crescent, Inchinnan Business Park, Inchinnan, Renfrew, PA4 9RE

US Suppliers

Equipment for the initial preparation of samples e.g. Tecator homogeniser available from Perstorp Analytical inc., 12101 Tech Road, Silver Spring, Maryland 20904.

General laboratory glassware, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Plastic centrifuge bottles, 250 mL size, available from Fisher Scientific UK, Liberty Lane, Hampton, NH 03842, USA.

High speed homogeniser for extraction of samples e.g. Janke and Kunkel Ultra Turrax T25, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Laboratory centrifuge e.g. Heraeus Instruments model 17RS, available from Heraeus Instruments, 111-A Corporate Blvd, South Plainfield, NJ 07080, USA.

Disposable test tubes (10 mL capacity) available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Isolute Vacmaster-20™ sample processing station, available from Jones Chromatography USA Ltd., PO Box 280 329, Lakewood, Colorado, 8022-0329.

Isolute C18 (EC) solid phase extraction columns, 6 mL 1 g size, available from Fisher Scientific UK, Liberty Lane, Hampton, NH 03842, USA.

Techne Dri-block 3D sample concentrator, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Ultrasonic bath available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Crimp cap auto sampler vials and caps available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304 USA.

API3000 HPLC-MS-MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128, USA.

HPLC column, KR100 5C18 50 mm x 3.2 mm i.d., 5 μm particle size, available from www.hichrom.co.uk

Agilent 1100 HPLC system equipped with a quaternary pump, vacuum degasser and column compartment with column switching valve, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304 USA.

CTC HTS PAL autosampler, available from LEAP Technologies Inc., P.O. Box 969, Carrboro, NC 27510

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments, 1300 West Belmont Ave, Chicago, Il 60657

Appendix 2 : Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used.

UK Suppliers

Acetonitrile, dichloromethane, ethyl acetate, methanol, hexane super purity grade and bottled HPLC grade water, available from Rathburn Chemicals Ltd., Walkerburn, Scotland EH43 6AU, UK.

Acetic Acid, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Ultra pure water from a laboratory water purification system e.g. Elga Maxima available from Elga Ltd., High Street, Lane End, High Wycombe, Buckinghamshire HP14 3JH, UK.

Azoxystrobin and R230310 analytical standards available from Syngenta, GLP Testing Facility EZA, Syngenta, CH-4333 Munchwilen, Switzerland

US Suppliers

Acetonitrile, dichloromethane, ethyl acetate, methanol, hexane super purity grade and bottled HPLC grade water available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA (Tel: 312-689-8410).

Acetic acid, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Ultra-pure water from a laboratory water purification system available from Waters Corporation, Milford, MA, USA.

Azoxystrobin and R230310 analytical standard available from Syngenta Crop Protection Inc. P.O. Box 18300, Greensboro, NC 27419-8300

Appendix 3 : Method Validation Data

Table 1: Recovery Data For Azoxystrobin Primary Transition, *m/z* 404.2 → 372.4, Obtained During Method Validation

Matrix	Fortification Level (mg kg^{-1})	Recovery (%)	*Mean (%)	RSD (%)	Range (%)
Beer	0.01	97, 86, 61, 86, 91	84	16	61 - 97
	0.10	84, 89, 85, 90, 87	87	3	84 - 90
	Overall		86	11	61 - 97
Mandarin	0.01	102, 97, 100, 100, 95	99	3	95 - 100
	10	98, 98, 99, 96, 98	98	1	96 - 99
	Overall		98	2	95 - 100
Cabbage	0.01	87, 85, 83, 91, 87	86	3	83 - 91
	0.3	92, 96, 92, 94, 92	93	2	92 - 96
	Overall		90	5	83 - 96
Wheat Grain	0.01	94, 102, 88, 84, 91	92	7	84 - 102
	0.3	112, 98, 99, 97, 99	101	6	97 - 112
	Overall		96	8	84 - 112
Wheat Straw	0.01	99, 83, 81, 87, 83	87	8	81 - 99
	7.5	90, 85, 87, 90, 87	88	2	85 - 90
	Overall		87	6	81 - 99
Wheat Flour	0.01	90, 85, 87, 93, 97	90	5	85 - 97
	0.10	91, 94, 96, 92, 94	94	2	91 - 96
	Overall		92	4	85 - 97
Sunflower Seed	0.01	90, 88, 114, 97, 90	96	11	88 - 114
	0.5	81, 94, 96, 90, 89	90	6	81 - 96
	Overall		93	9	81 - 114

* The mean (%) response was calculated before rounding the recovery (%) values.

Table 2: Recovery Data For R230310 Primary Transition, *m/z* 404.2 → 372.4, Obtained During Method Validation

Matrix	Fortification Level (mg kg^{-1})	Recovery (%)	*Mean (%)	RSD (%)	Range (%)
Beer	0.01	82, 86, 58, 85, 78	78	14	58 - 86
	0.10	84, 87, 86, 92, 89	88	4	84 - 92
	Overall		87	9	58 - 92
Mandarin	0.01	98, 99, 96, 103, 101	99	3	96 - 103
	10	92, 92, 90, 89, 88	90	2	88 - 92
	Overall		95	6	88 - 103
Cabbage	0.01	91, 88, 87, 94, 91	90	3	87 - 94
	0.3	93, 95, 89, 90, 90	91	3	89 - 95
	Overall		90	3	87 - 95
Wheat Grain	0.01	96, 102, 89, 92, 92	94	5	89 - 102
	0.3	96, 97, 98, 92, 101	97	3	92 - 101
	Overall		95	4	89 - 102
Wheat Straw	0.01	91, 89, 89, 86, 84	88	3	84 - 91
	7.5	85, 85, 88, 88, 85	86	2	85 - 88
	Overall		87	3	84 - 91
Wheat Flour	0.01	88, 96, 88, 86, 93	90	5	86 - 96
	0.10	89, 94, 96, 94, 96	94	3	89 - 96
	Overall		92	4	86 - 96
Sunflower Seed	0.01	97, 89, 95, 101, 94	95	5	89 - 101
	0.5	86, 96, 93, 86, 91	90	5	86 - 96
	Overall		93	5	86 - 101

* The mean (%) response was calculated before rounding the recovery (%) values.

Table 3: Recovery Data For Azoxystrobin Confirmatory Transition, *m/z* 404.2 → 343.8, Obtained During Method Validation

Matrix	Fortification Level (mg kg^{-1})	Recovery (%)	*Mean (%)	RSD (%)	Range (%)
Beer	0.01	99, 95, 76, 97, 89	91	10	76 - 99
	0.10	85, 95, 90, 87, 89	89	4	87 - 95
	Overall		90	8	76 - 99
Mandarin	0.01	92, 99, 101, 97, 93	97	4	93 - 101
	10	97, 99, 99, 97, 97	98	1	97 - 99
	Overall		97	3	93 - 101
Cabbage	0.01	104, 86, 77, 101, 104	95	13	77 - 104
	0.3	94, 95, 90, 94, 94	93	2	90 - 95
	Overall		94	9	77 - 104
Wheat Grain	0.01	103, 102, 97, 92, 91	97	5	91- 103
	0.3	113, 95, 98, 96, 102	101	7.3	95 - 113
	Overall		99	6	91 - 113
Wheat Straw	0.01	110, 108, 95, 102, 108	105	6	95 - 110
	7.5	91, 88, 90, 93, 90	90	2	88 - 93
	Overall		97	9	88 - 110
Wheat Flour	0.01	102, 96, 102, 91, 111	100	7	91 - 111
	0.10	94, 95, 100, 100, 97	97	3	94 - 100
	Overall		99	6	91 - 111
Sunflower Seed	0.01	100, 87, 118, 103, 83	98	14	83 - 118
	0.5	82, 96, 97, 91, 90	91	7	82 - 97
	Overall		95	11	82 - 118

* The mean (%) response was calculated before rounding the recovery (%) values.

Table 4: Recovery Data For R230310 Confirmatory Transition, *m/z* 404.2 → 343.8, Obtained During Method Validation

Matrix	Fortification Level (mg kg^{-1})	Recovery (%)	*Mean (%)	RSD (%)	Range (%)
Beer	0.01	90, 91, 76, 91, 95	89	8	76 - 95
	0.10	83, 88, 93, 92, 91	90	5	83 - 93
	Overall		89	6	76 - 95
Mandarin	0.01	77, 76, 95, 102, 88	88	13	76 - 102
	10	93, 93, 91, 90, 88	91	2	88 - 93
	Overall		89	9	76 - 102
Cabbage	0.01	90, 80, 78, 87, 95,	86	8	78 - 95
	0.3	92, 94, 90, 91, 91	92	2	90 - 94
	Overall		89	6	78 - 95
Wheat Grain	0.01	101, 108, 95, 90, 90	97	8	95 - 108
	0.3	93, 95, 97, 91, 101	95	4	91 - 101
	Overall		96	6	91 - 108
Wheat Straw	0.01	88, 78, 85, 95, 78	85	9	78 - 95
	7.5	85, 85, 86, 89, 87	86	2	85 - 89
	Overall		86	6	78 - 95
Wheat Flour	0.01	83, 79, 98, 91, 108	92	13	79 - 108
	0.10	89, 93, 97, 92, 93	93	3	89 - 97
	Overall		92	9	79 - 108
Sunflower Seed	0.01	101, 95, 90, 97, 102	97	5	90 - 102
	0.5	91, 94, 93, 88, 90	91	3	88 - 94
	Overall		94	5	88 - 102

* The mean (%) response was calculated before rounding the recovery (%) values.

Appendix 4 : Representative Chromatograms

Figure 3: **0.0001 µg mL^{-1} Azoxystrobin Standard (primary transition *m/z* 404.2 → 372.4).**

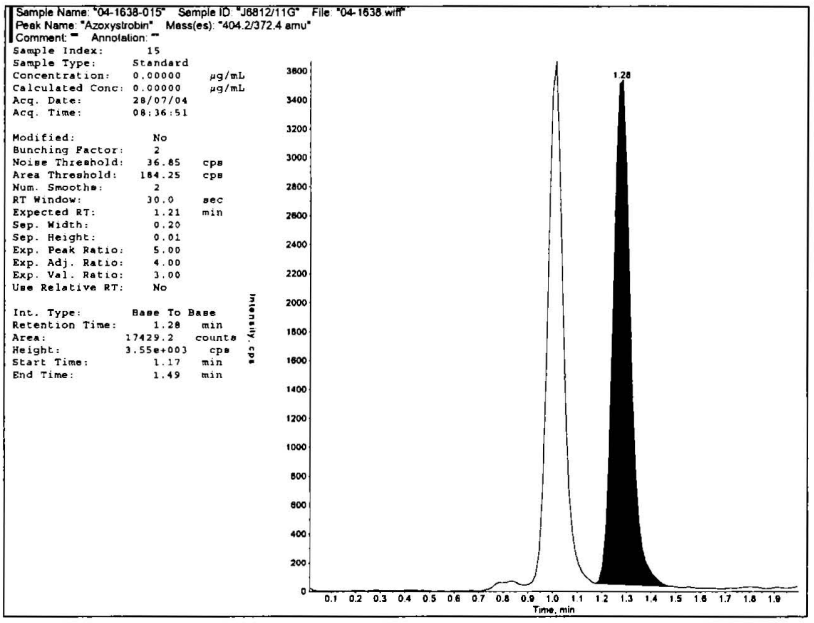


Figure 4: **0.0001 µg mL^{-1} Azoxystrobin Standard (confirmatory transition *m/z* 404.2 → 343.8).**

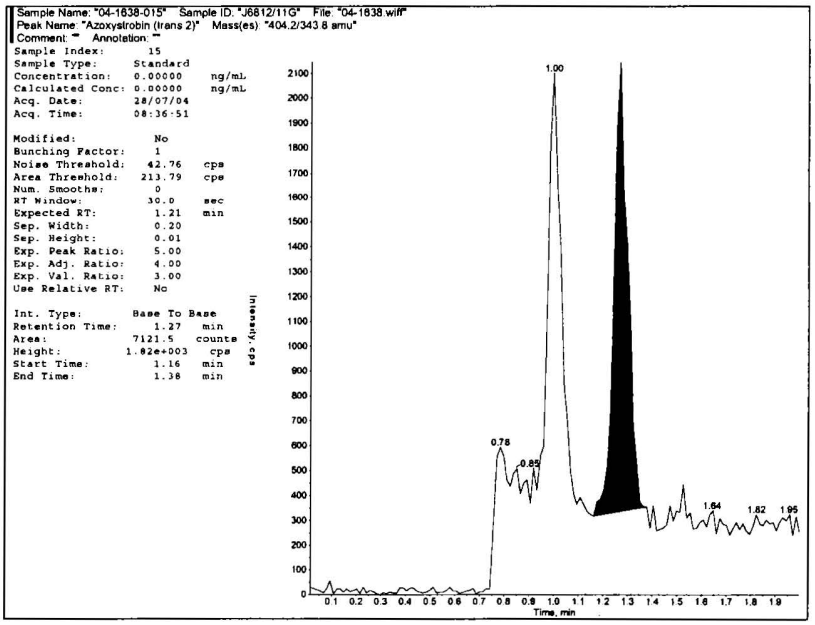


Figure 5: **0.0001 μg mL^{-1} R230310 Standard (primary transition *m/z* 404.2 → 372.4).**

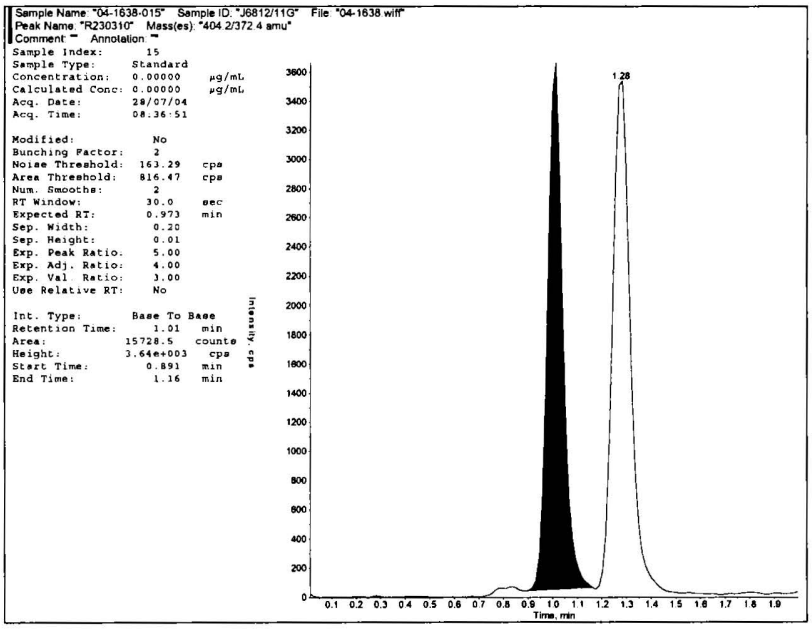


Figure 6: **0.0001 μg mL^{-1} R230310 Standard (confirmatory transition *m/z* 404.2 → 343.8).**

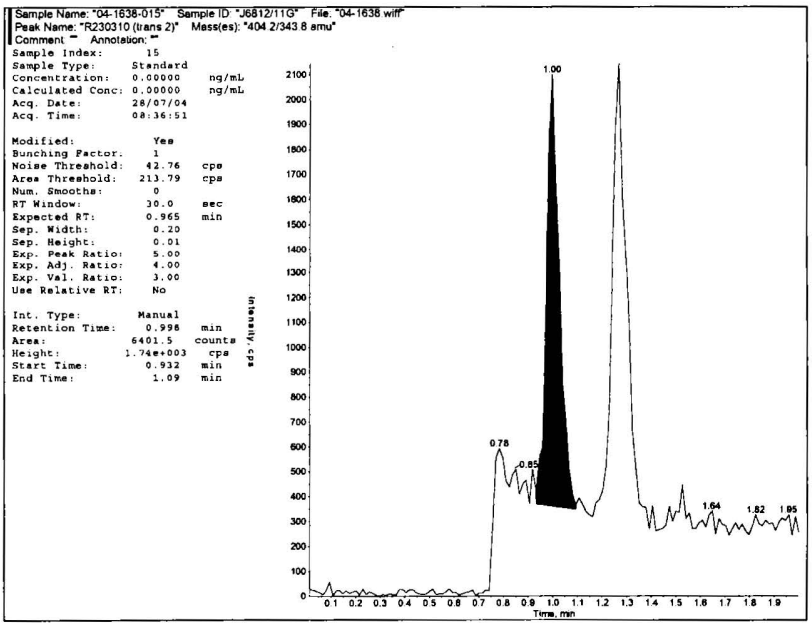


Figure 7: **0.05 μg mL^{-1} Azoxystrobin Standard primary transition (*m/z* 404.2 → 372.4).**

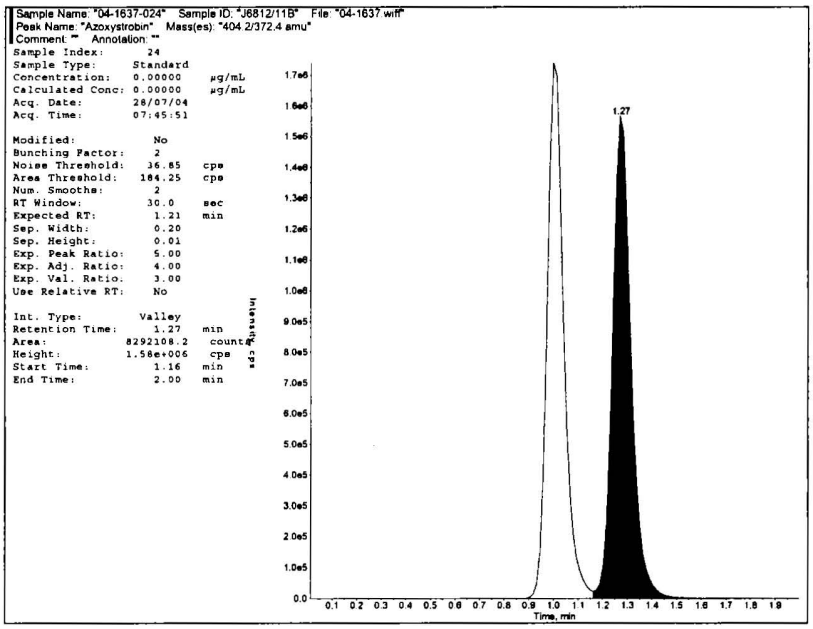


Figure 8: **0.05 μg mL^{-1} Azoxystrobin Standard confirmatory transition (*m/z* 404.2 → 343.8).**

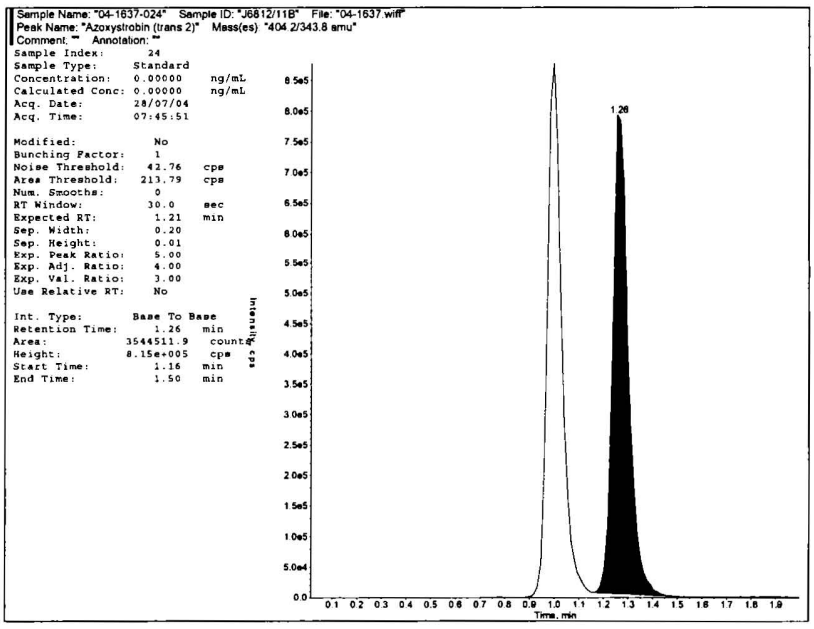


Figure 9: **0.05 μg mL^{-1} R230310 Standard primary transition (*m/z* 404.2 → 372.4).**

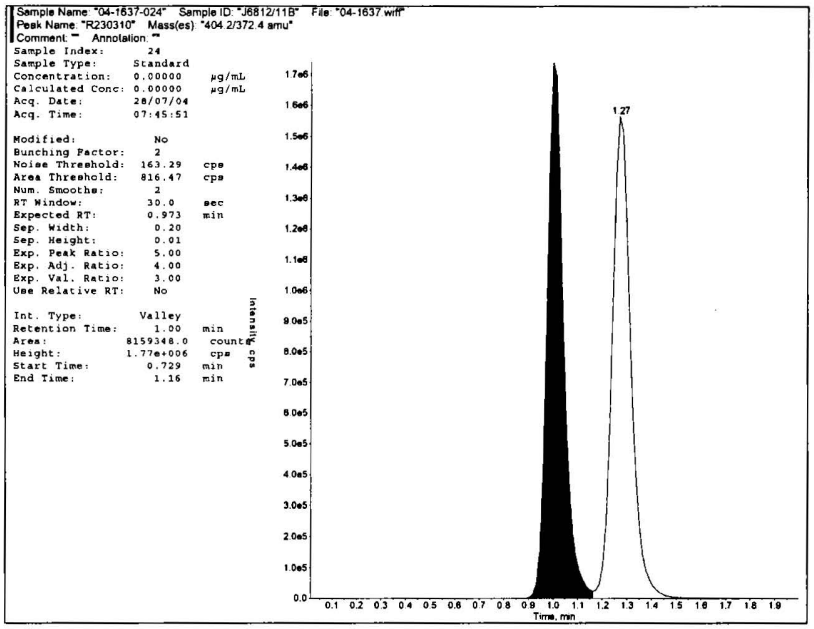


Figure 10: **0.05 μg mL^{-1} R230310 Standard confirmatory transition (*m/z* 404.2 → 343.8).**

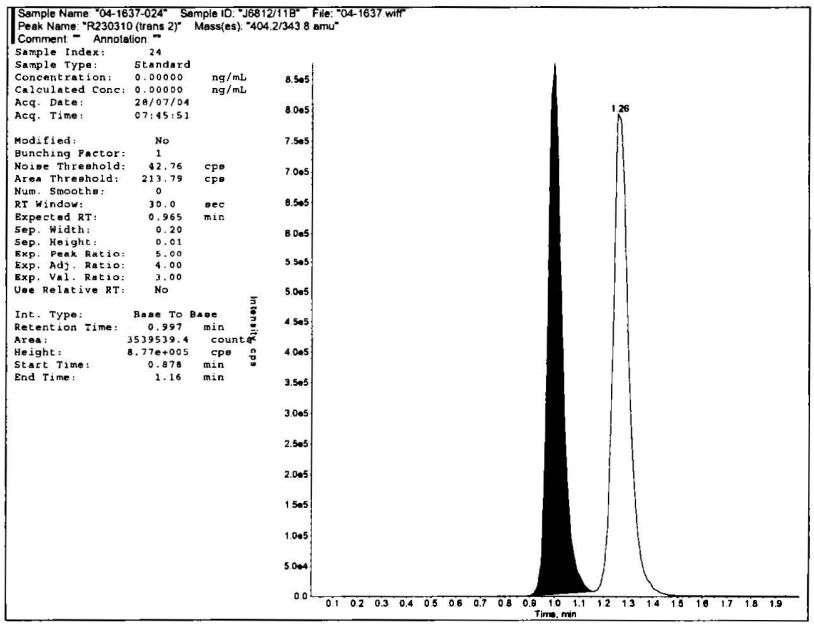


Figure 11: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Sample 04-S616 3/0. Azoxystrobin (primary transition) residue = <0.01 mg kg^{-1}.**

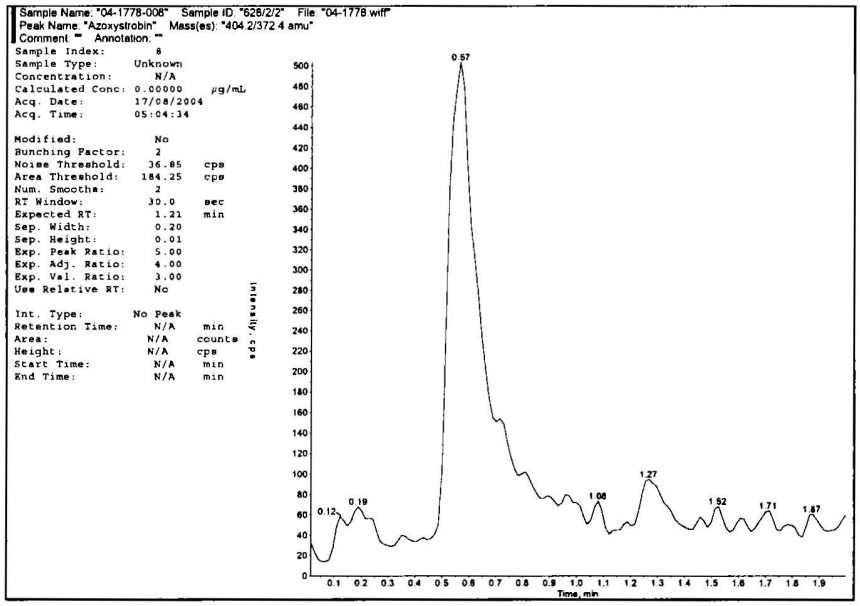


Figure 12: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Sample 04-S616 3/0. Azoxystrobin (confirmatory transition) residue = <0.01 mg kg^{-1}.**

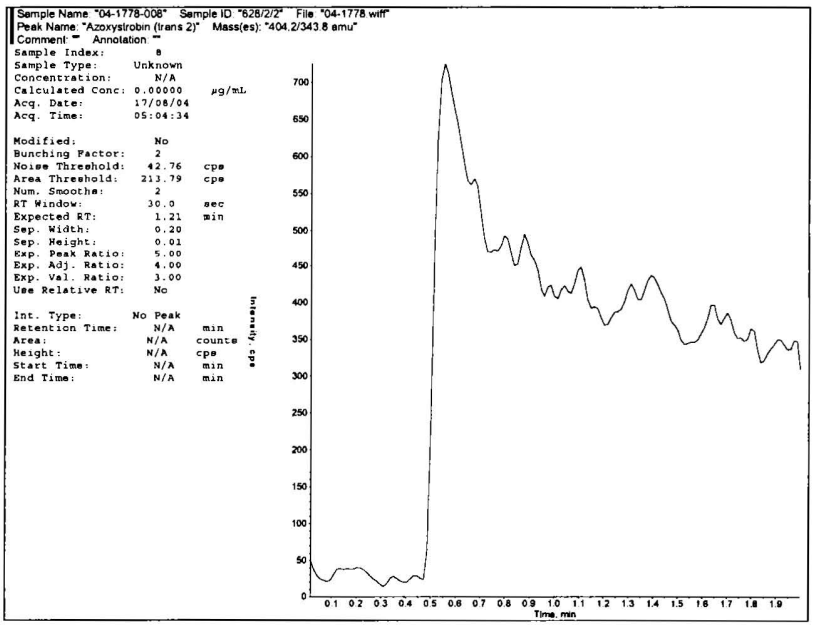


Figure 13: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Sample 04-S616 3/0. R230310 (primary transition) residue = <0.01 mg kg^{-1}.**

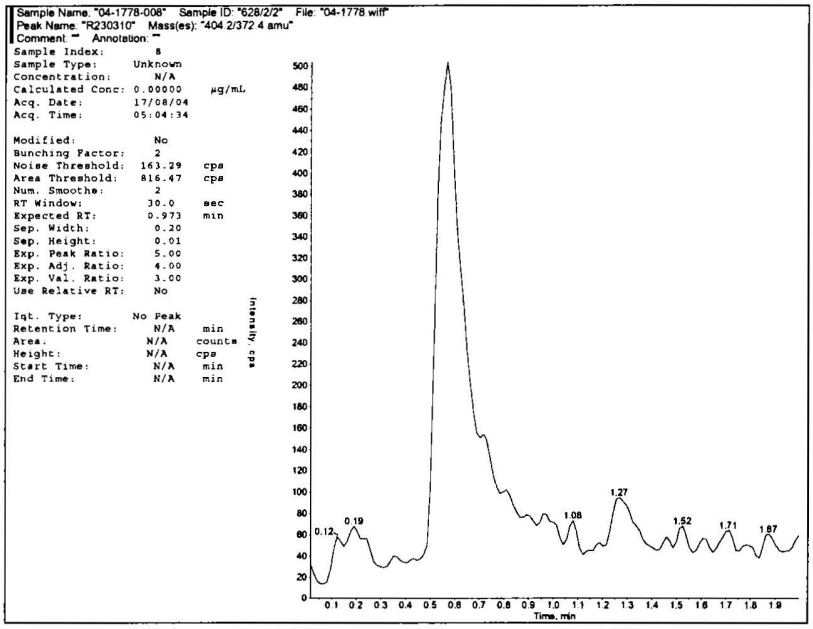


Figure 14: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Sample 04-S616 3/0. R230310 (confirmatory transition) residue = <0.01 mg kg^{-1}.**

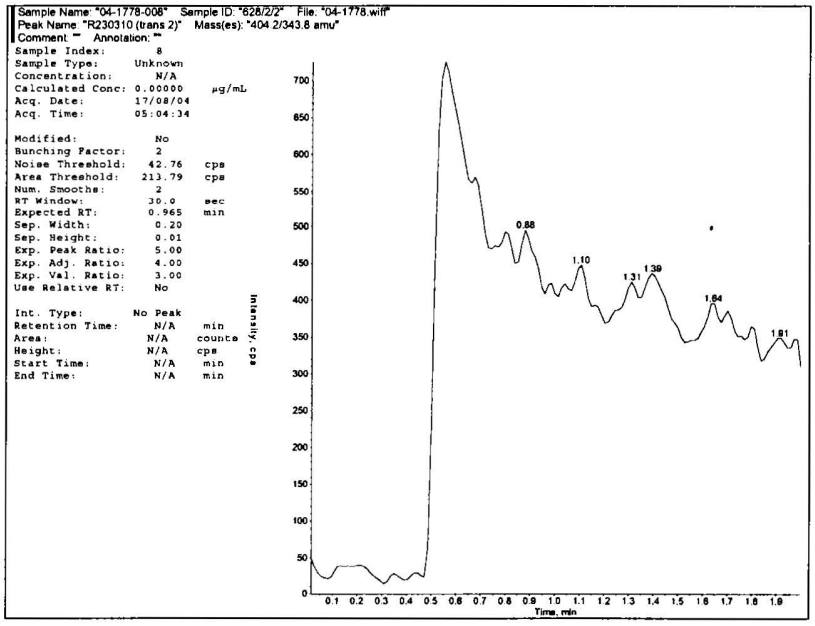


Figure 15: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 91%**

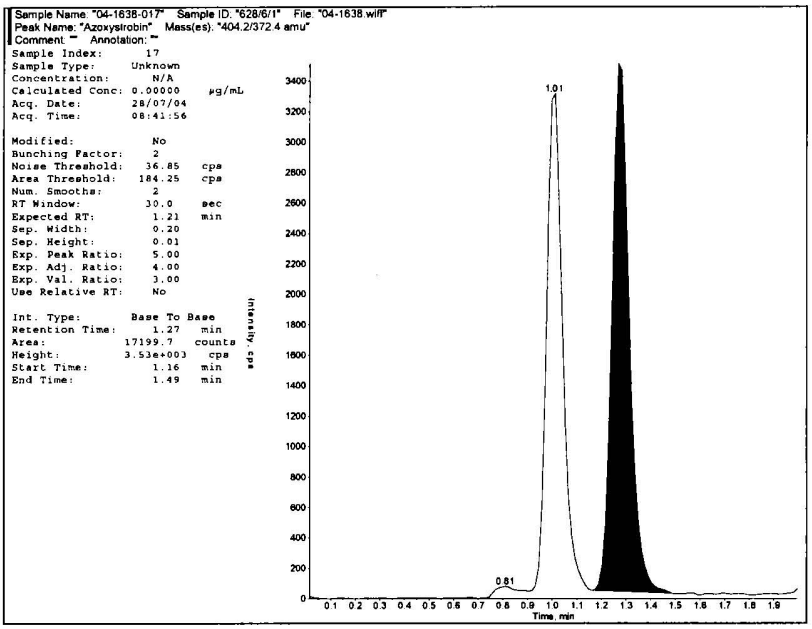


Figure 16: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 101%**

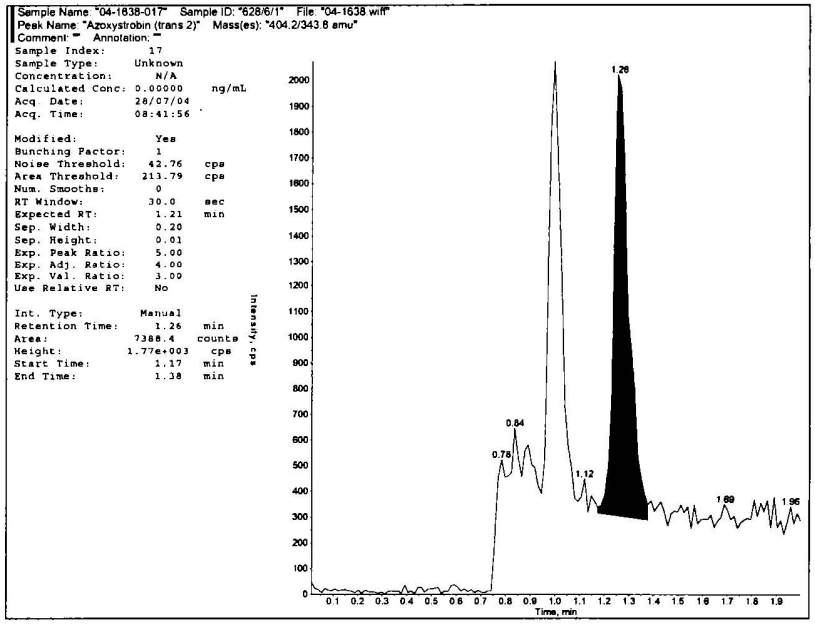


Figure 17: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. R230310 (primary transition) recovery = 94%**

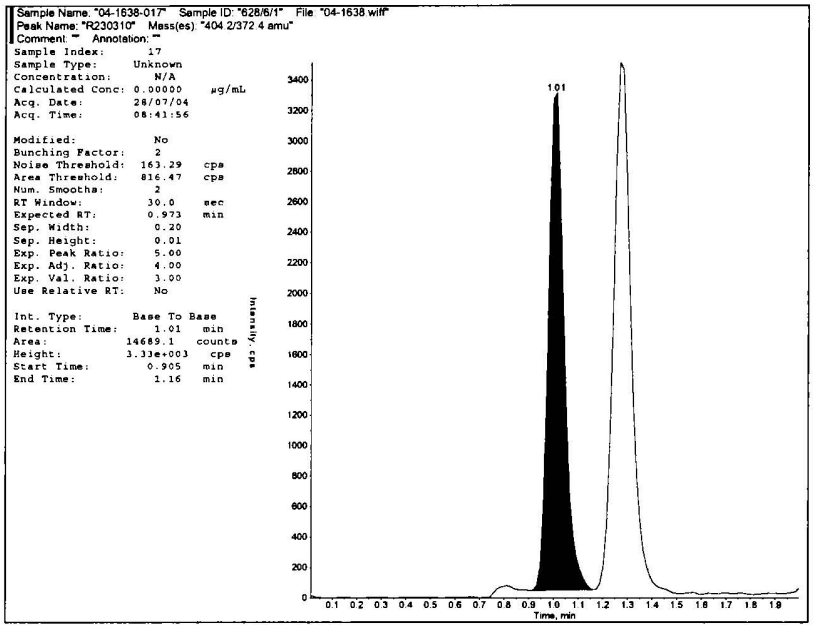


Figure 18: **Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}.R230310 (confirmatory transition) recovery = 87%**

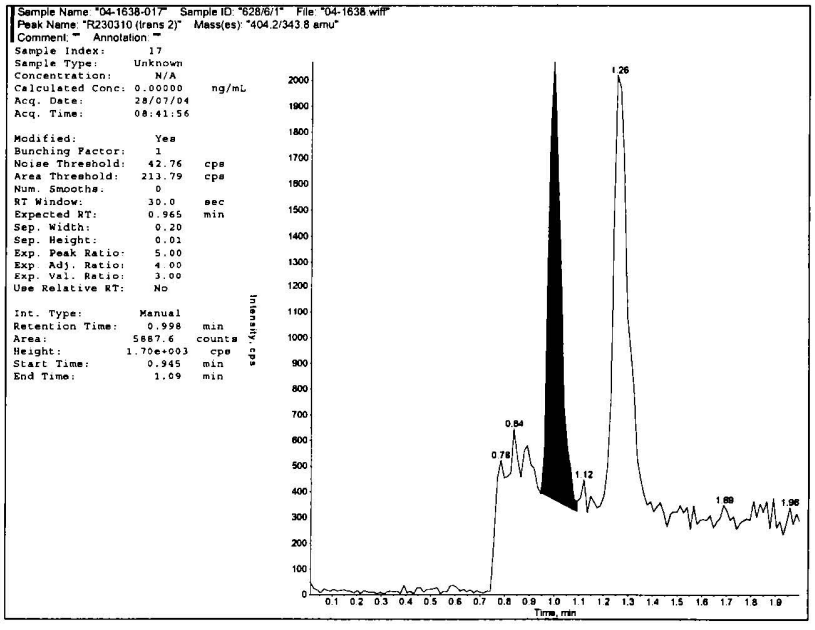


Figure 19: Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 96%

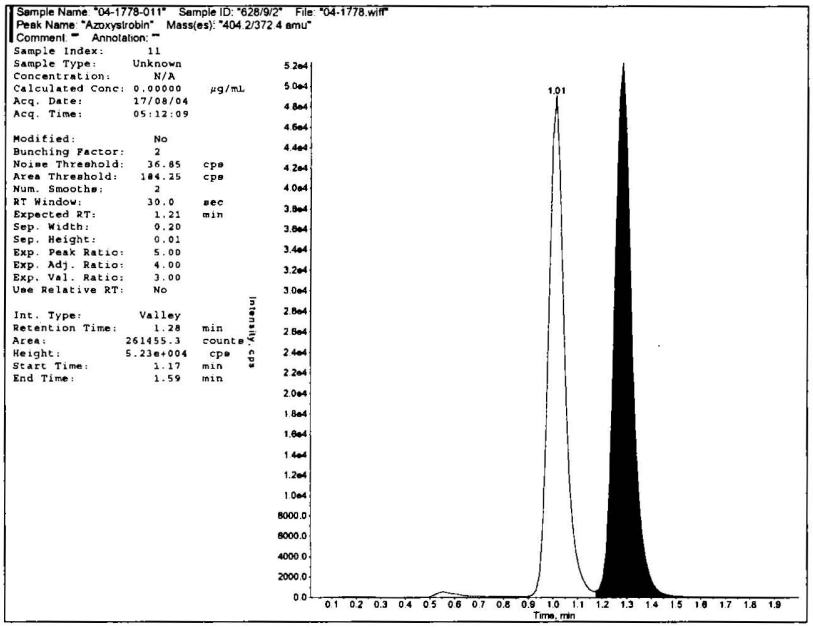


Figure 20: Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 95%

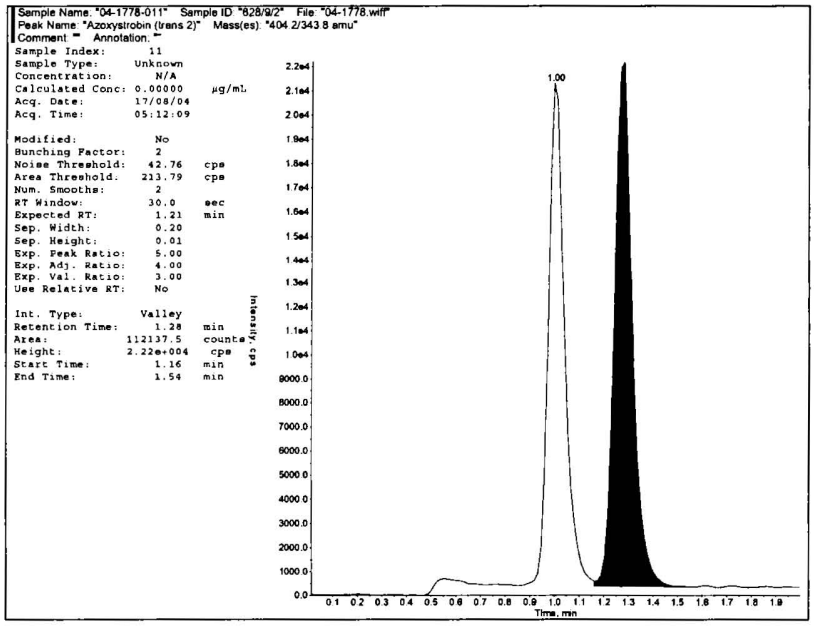


Figure 21: Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. R230310 (primary transition) recovery = 95%

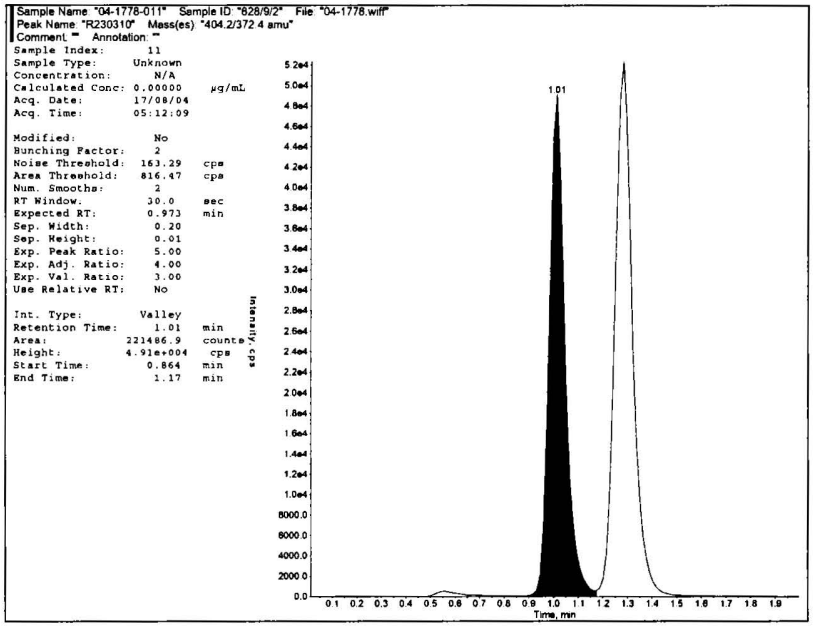


Figure 22: Untreated Sample of Cabbage at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. R230310 (confirmatory transition) recovery = 94%

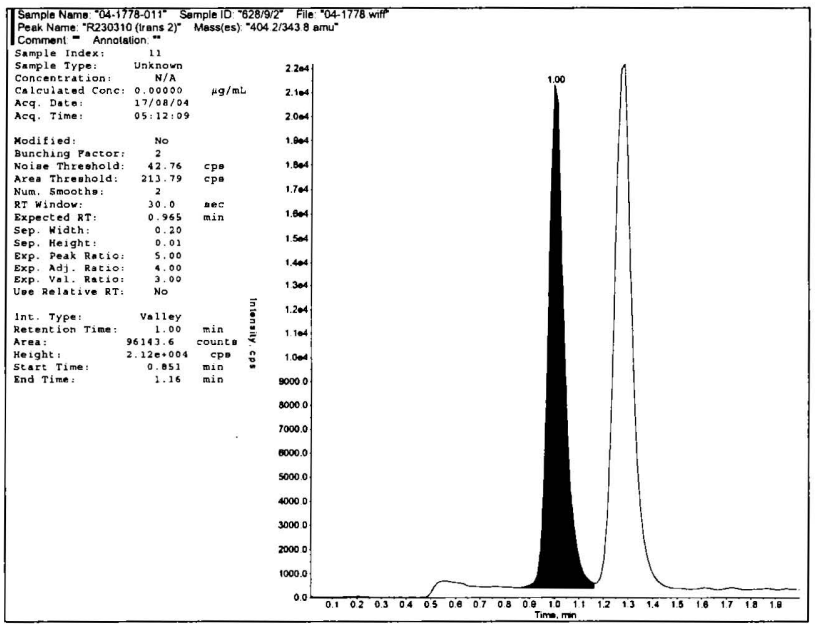


Figure 23: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Sample 04-S616 5/0. Azoxystrobin (primary transition) residue = <0.01 mg kg^{-1}.

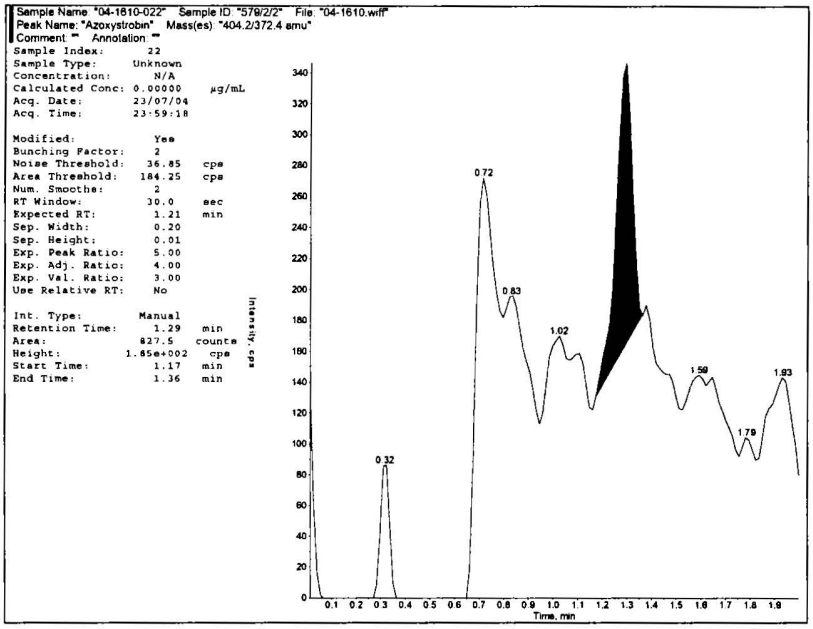


Figure 24: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Sample 04-S616 5/0. Azoxystrobin (confirmatory transition) residue = <0.01 mg kg^{-1}.

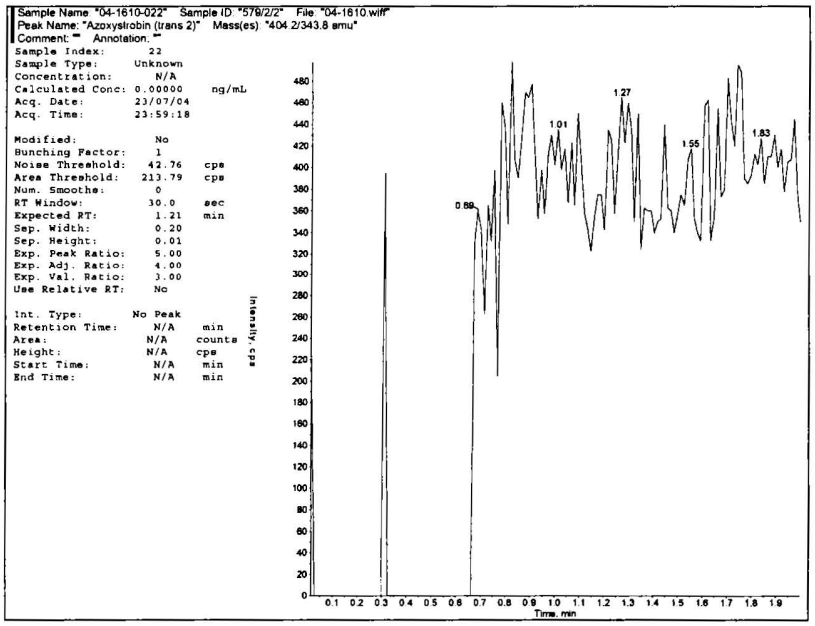


Figure 25: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Sample 04-S616 5/0. R230310 (primary transition) residue = <0.01 mg kg^{-1}.

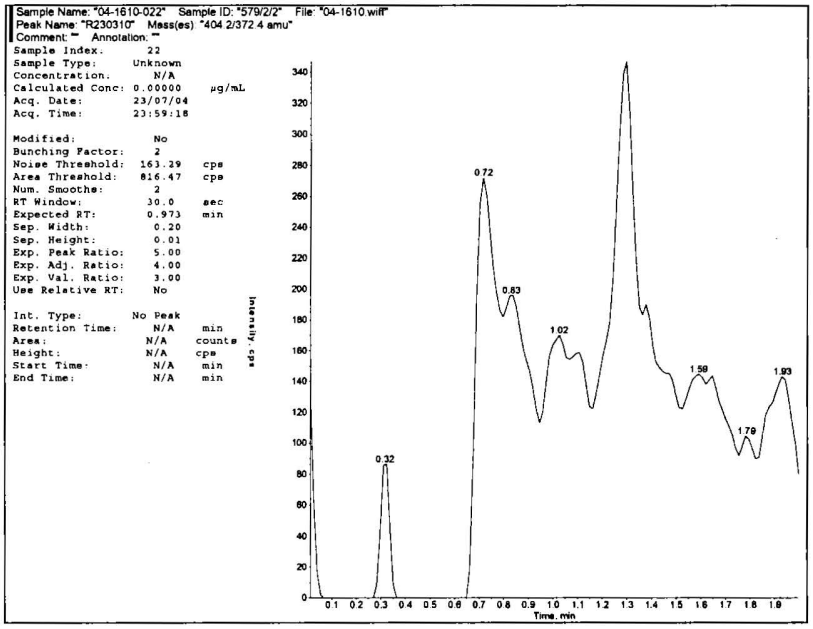


Figure 26: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Sample 04-S616 5/0. R230310 (confirmatory transition) residue = <0.01 mg kg^{-1}.

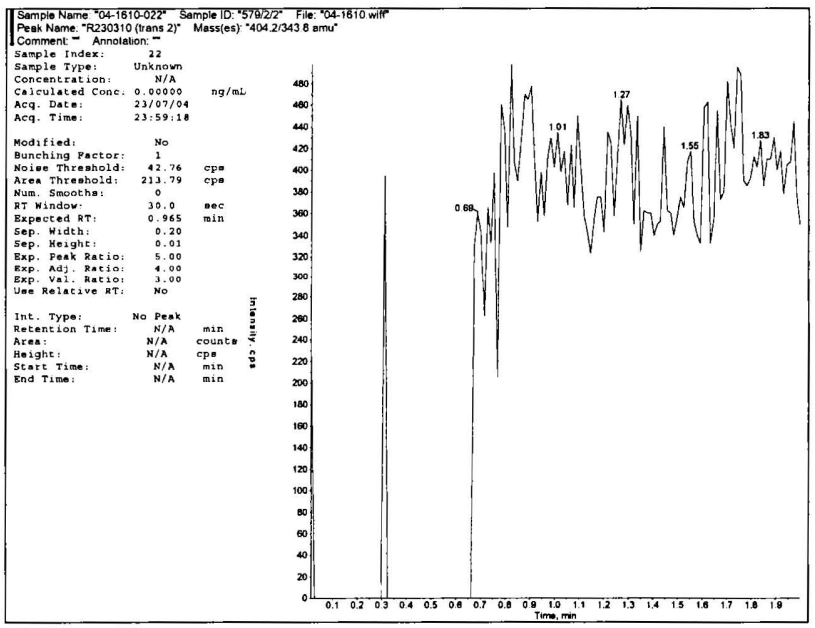


Figure 27: **Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 91%**

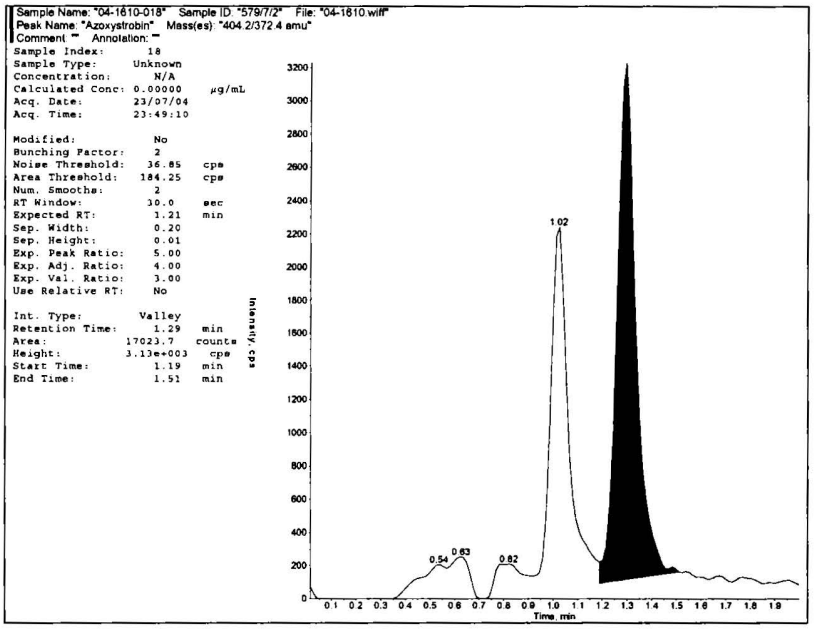


Figure 28: **Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 91%**

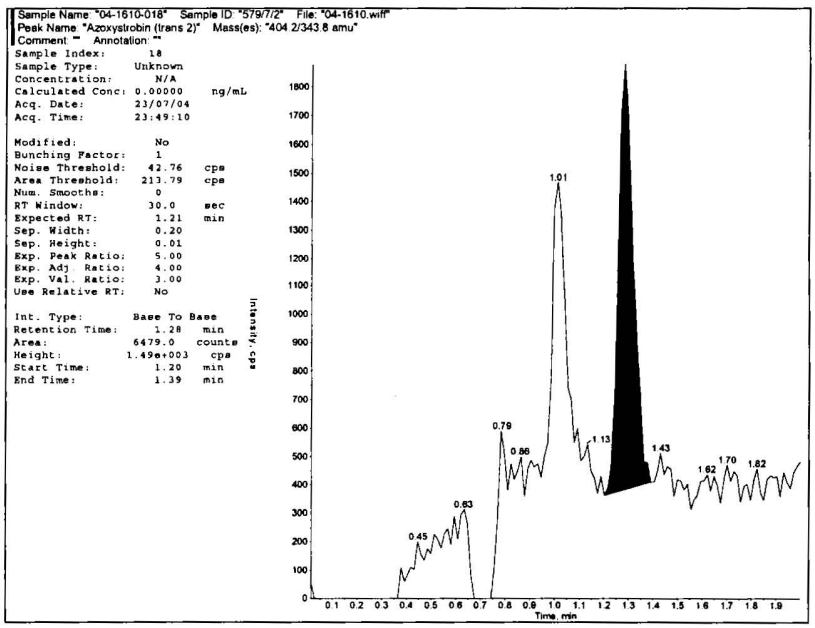


Figure 29: **Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. R230310 (primary transition) recovery = 92%**

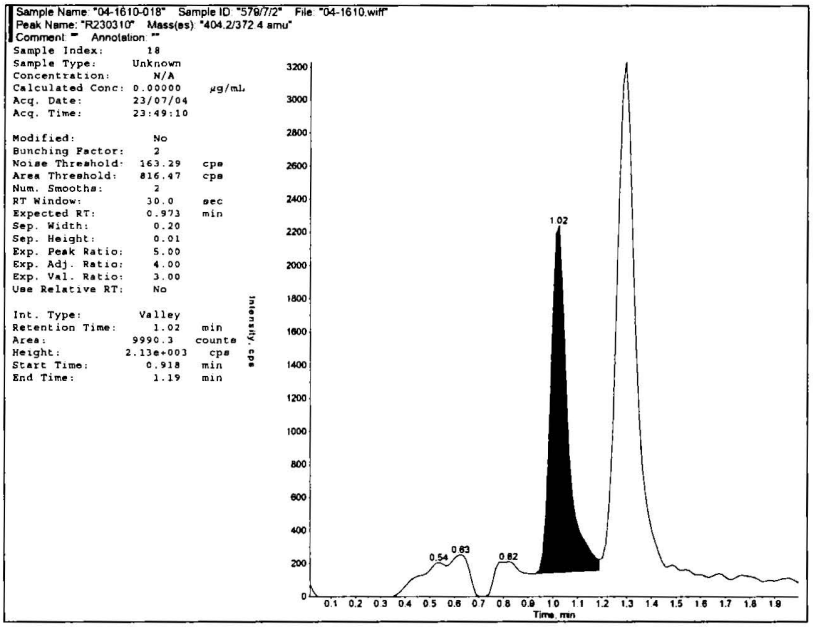


Figure 30: **Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. R230310 (confirmatory transition) recovery = 90%**

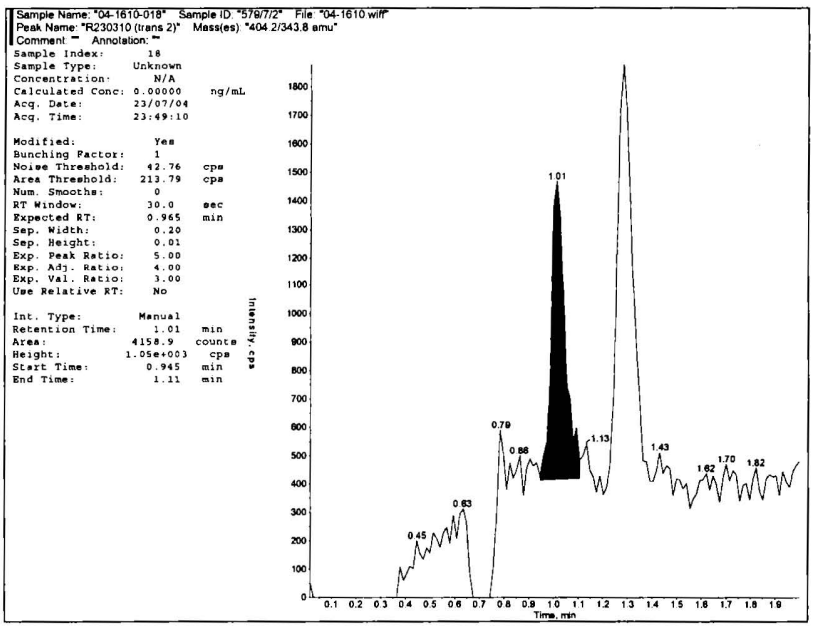


Figure 31: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 99%

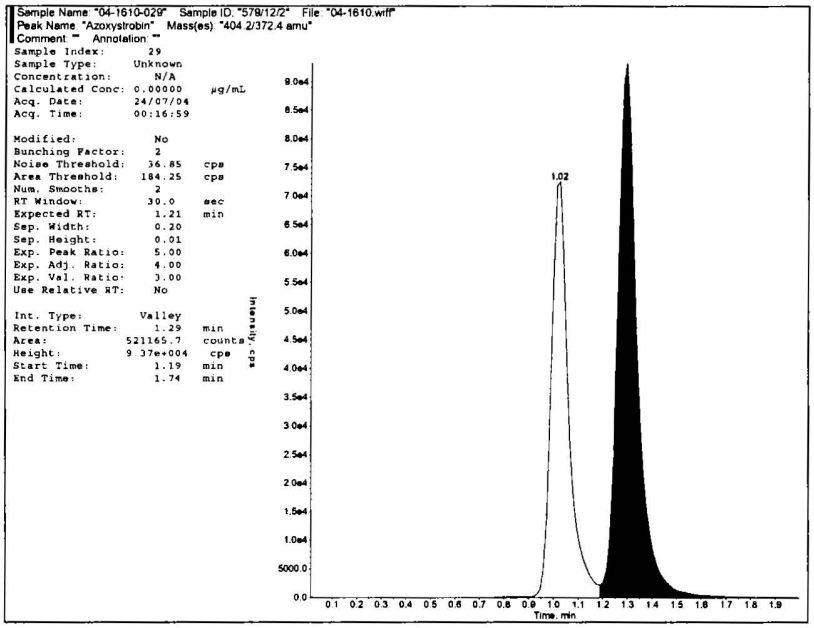


Figure 32: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 102%

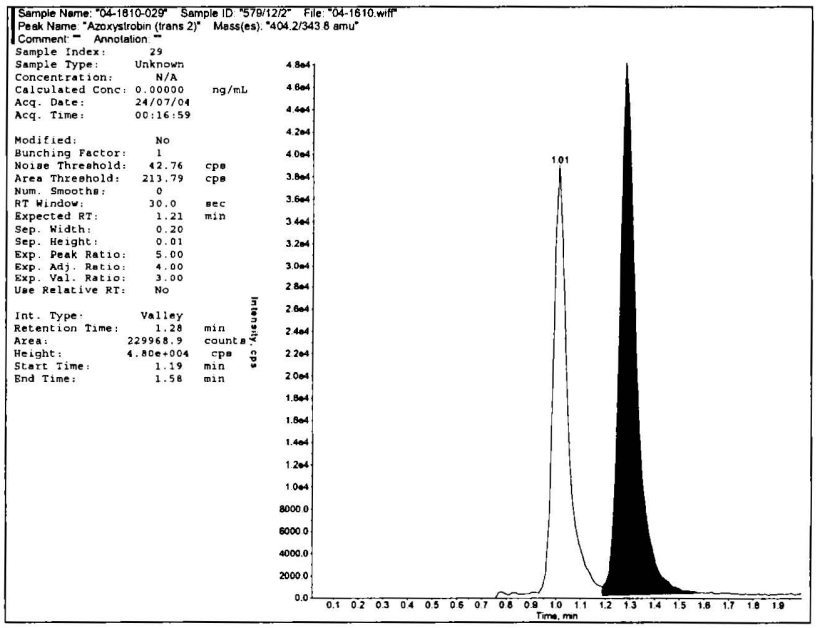


Figure 33: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. R230310 (primary transition) recovery = 101%

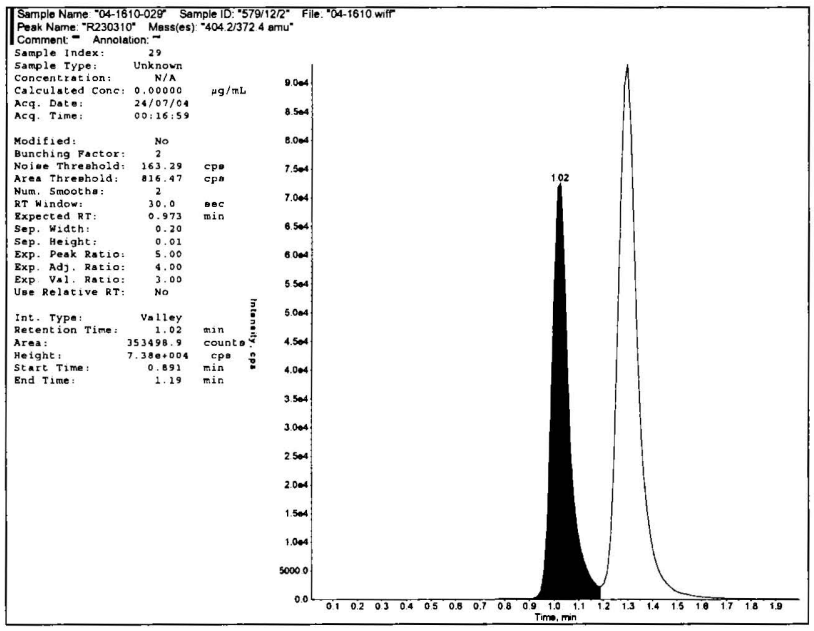


Figure 34: Untreated Sample of Wheat Grain at 0.01 g mL^{-1}. Fortified at 0.3 mg kg^{-1}. R230310 (confirmatory transition) recovery = 101%

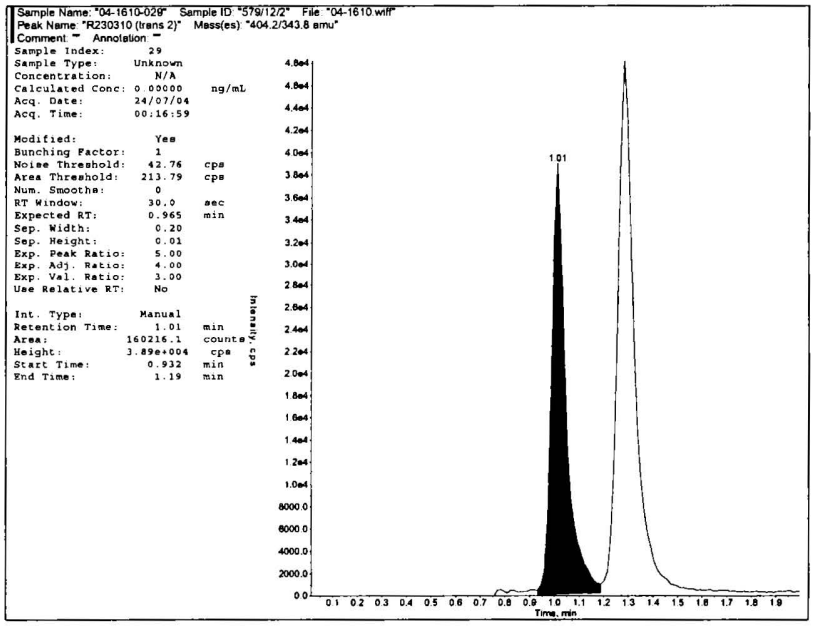


Figure 35: Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Sample 04-S616 8/0. Azoxystrobin (primary transition) residue = <0.01 mg kg^{-1}.

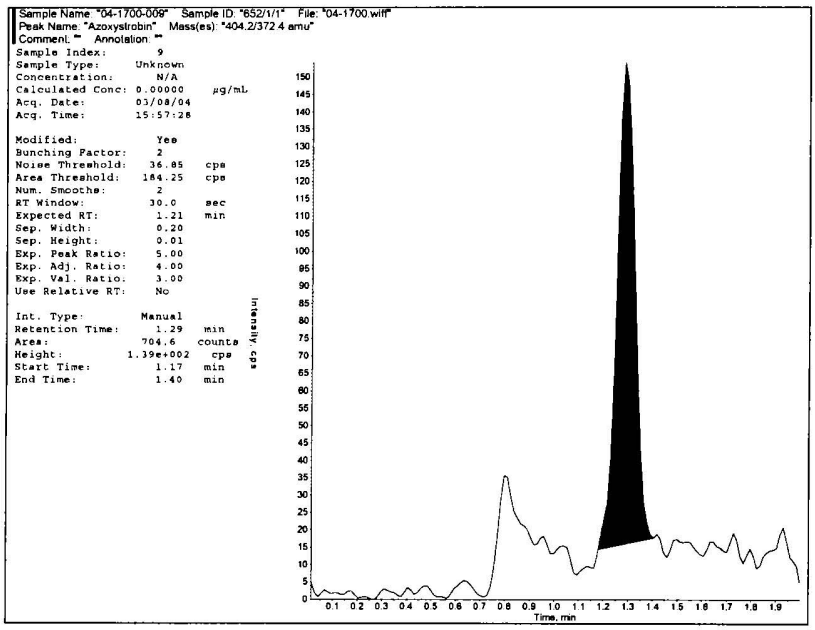


Figure 36: Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Sample 04-S616 8/0. Azoxystrobin (confirmatory transition) residue = <0.01 mg kg^{-1}.

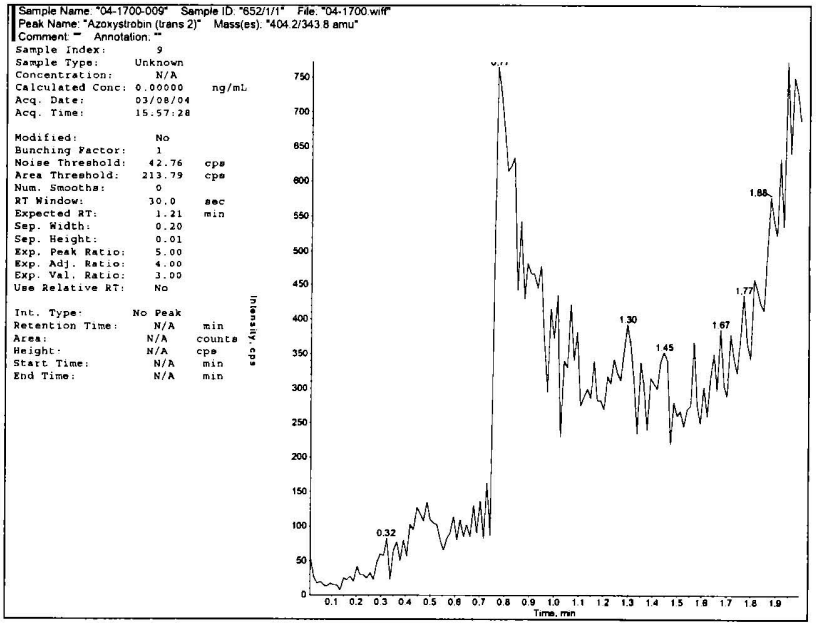


Figure 37: **Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Sample 04-S616 8/0. R230310 (primary transition) residue = <0.01 mg kg^{-1}.**

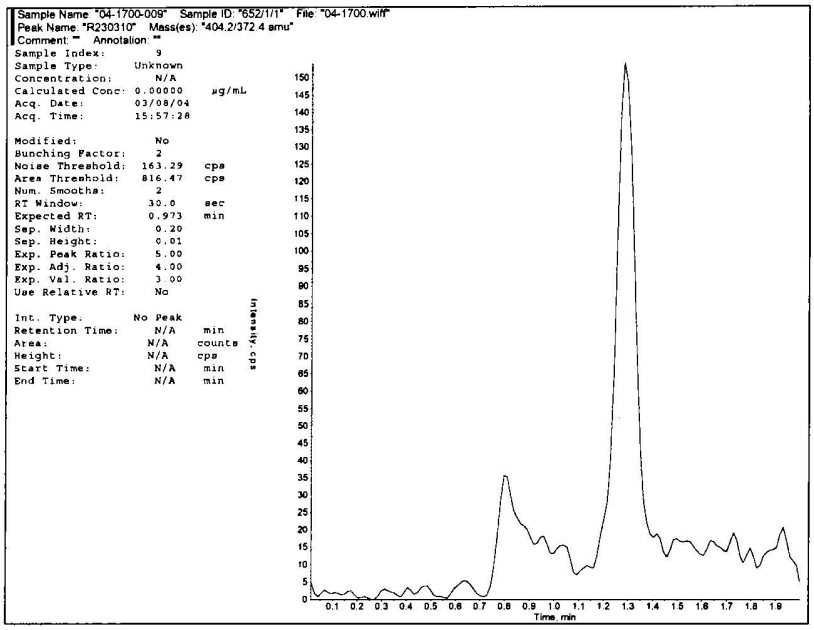


Figure 38: **Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Sample 04-S616 8/0. R230310 (confirmatory transition) residue = <0.01 mg kg^{-1}.**

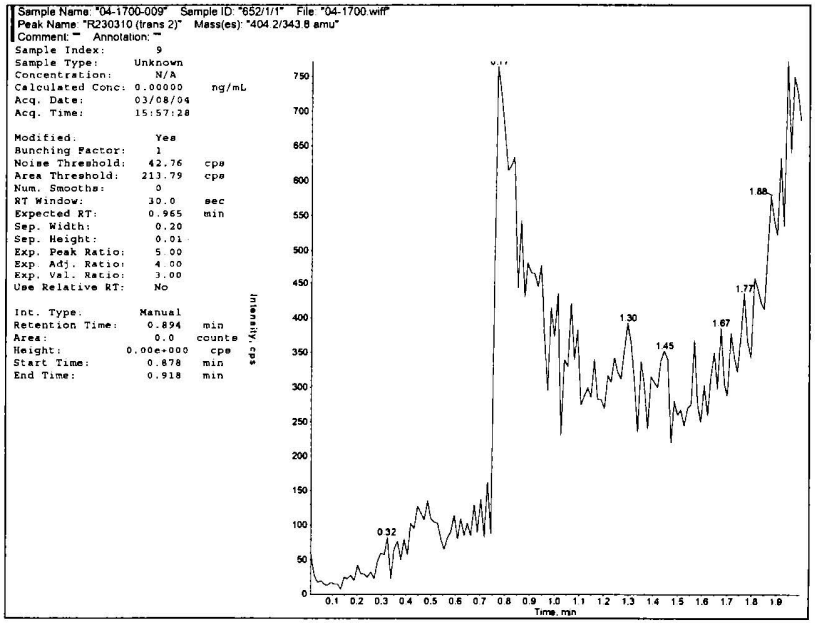


Figure 39: Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 90%

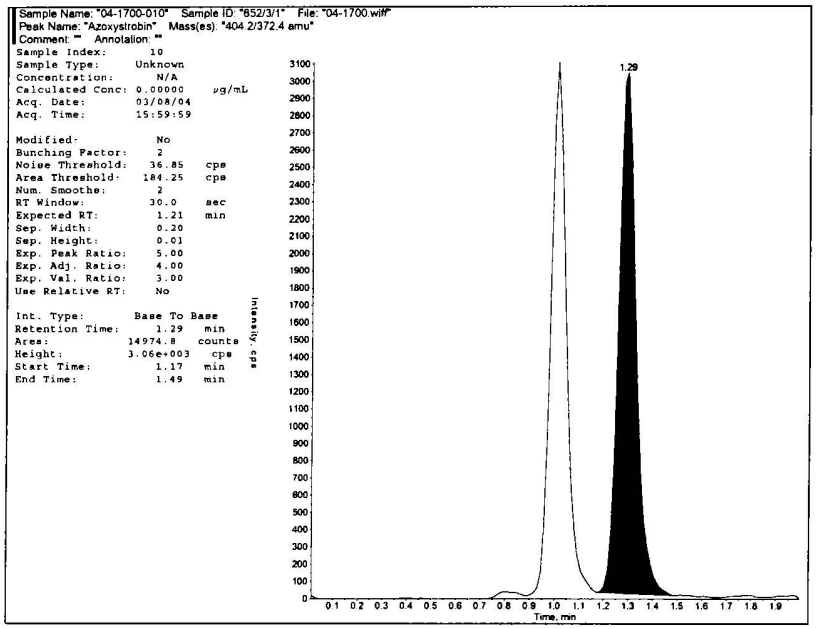


Figure 40: Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 100%

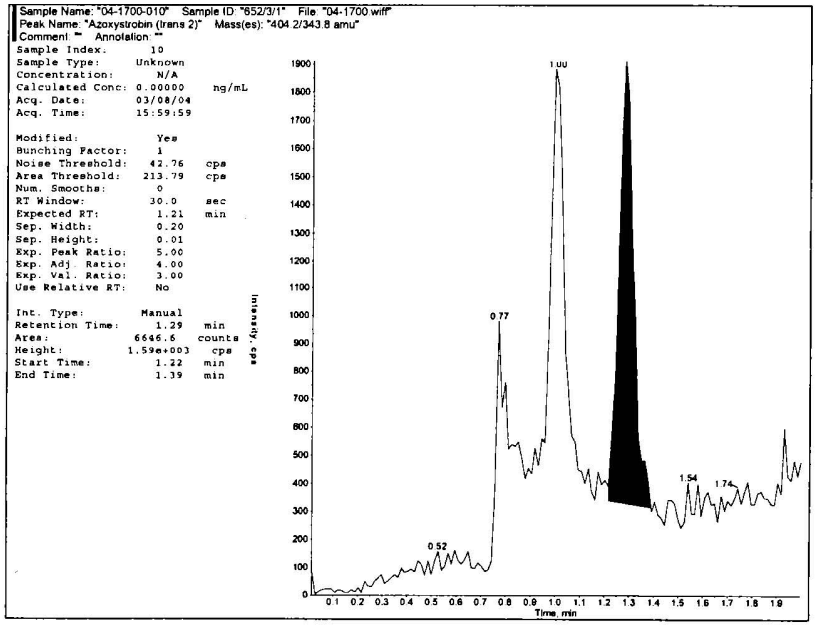


Figure 41: **Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. R230310 (primary transition) recovery = 97%**

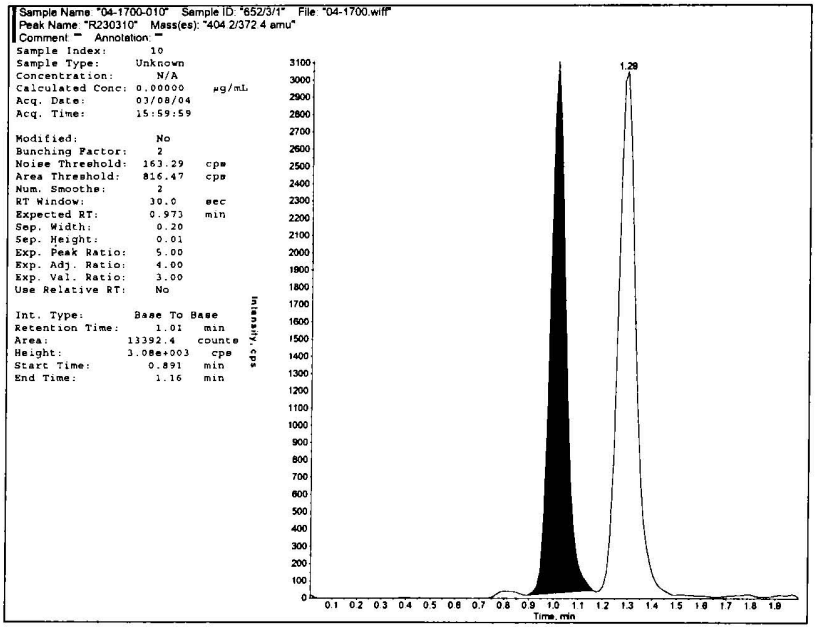


Figure 42: **Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. R230310 (confirmatory transition) recovery = 101%**

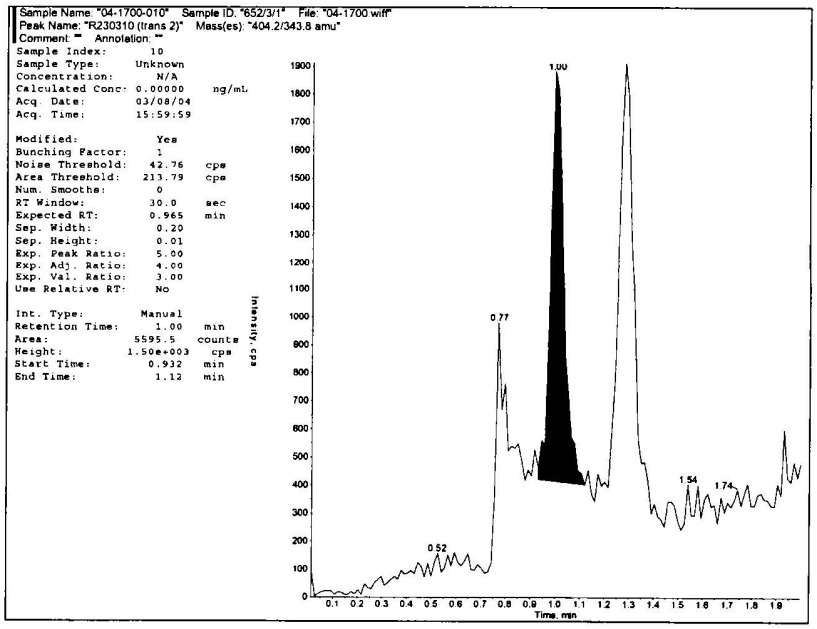


Figure 43: Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.5 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 89%

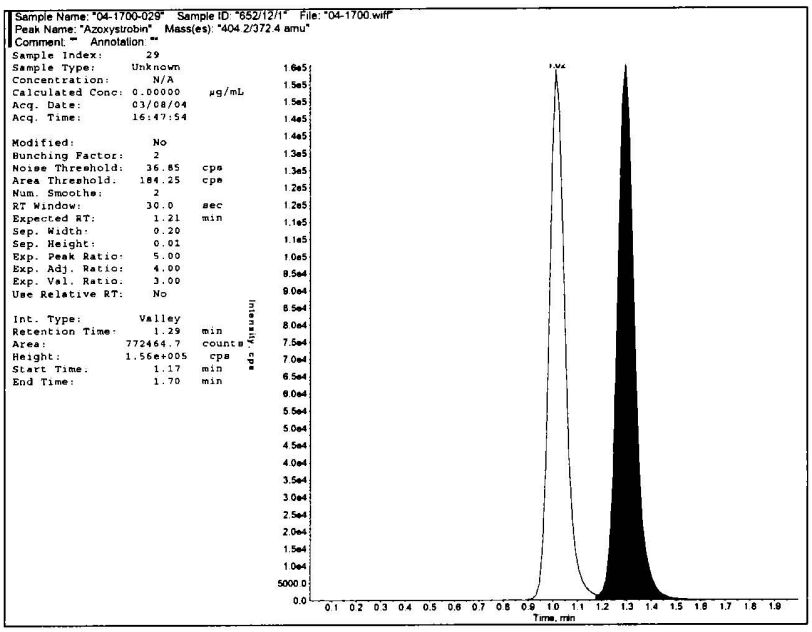


Figure 44: Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.5 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 91%

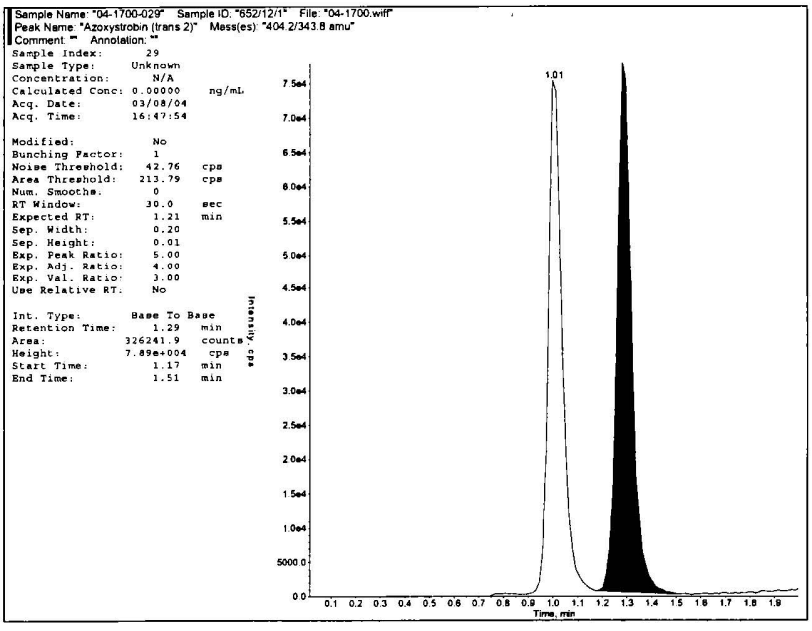


Figure 45: **Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.5 mg kg^{-1}. R230310 (primary transition) recovery = 90%**

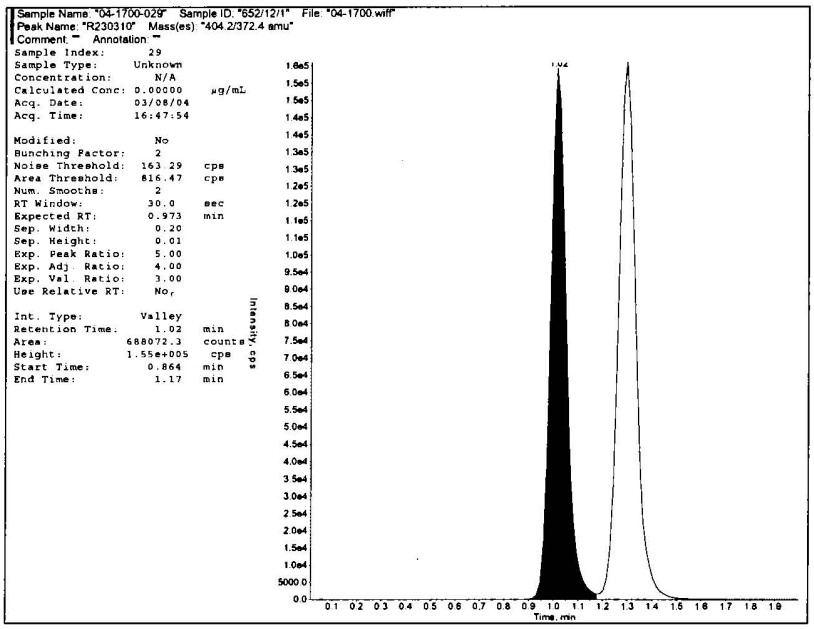


Figure 46: **Untreated Sample of Sunflower Seed at 0.01 g mL^{-1}. Fortified at 0.5 mg kg^{-1}. R230310 (confirmatory transition) recovery = 91%**

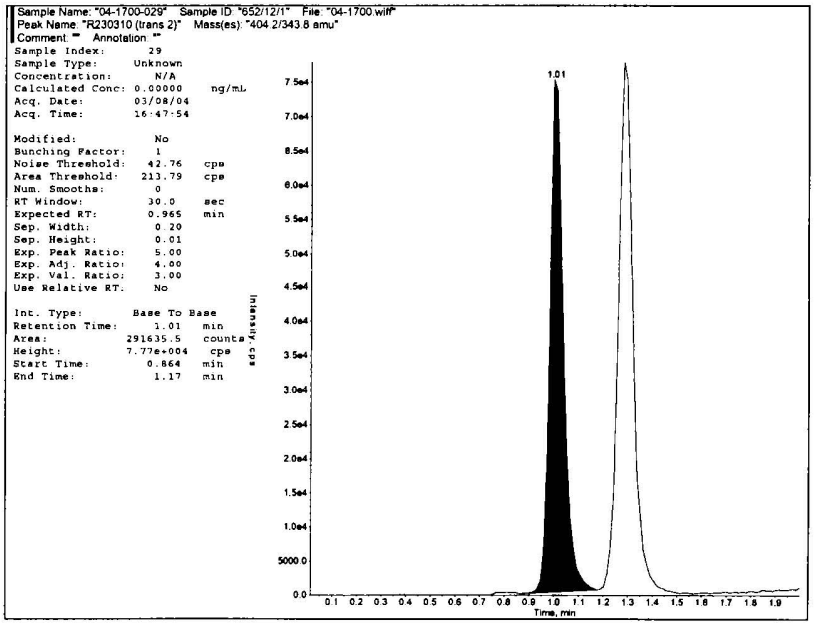


Figure 47: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Sample 04-S616 9/0. Azoxystrobin (confirmatory transition) residue = <0.01 mg kg^{-1}.

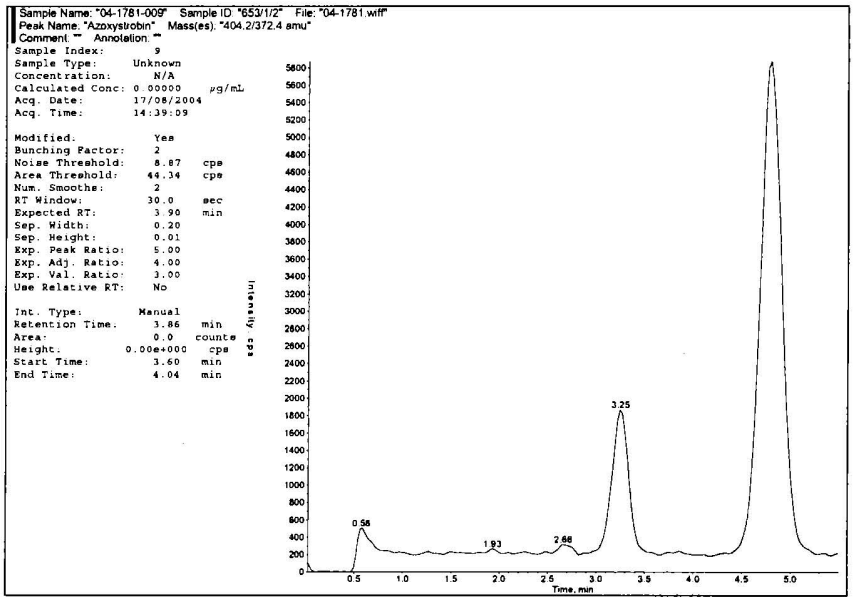


Figure 48: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Sample 04-S616 9/0. Azoxystrobin (confirmatory transition) residue = <0.01 mg kg^{-1}.

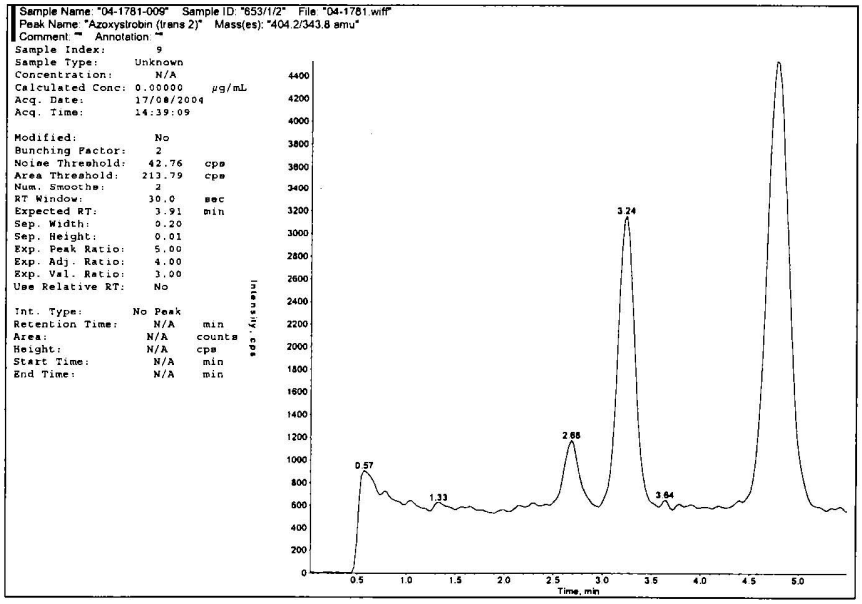


Figure 49: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Sample 04-S616 9/0. R230310 (primary transition) residue = <0.01 mg kg^{-1}.

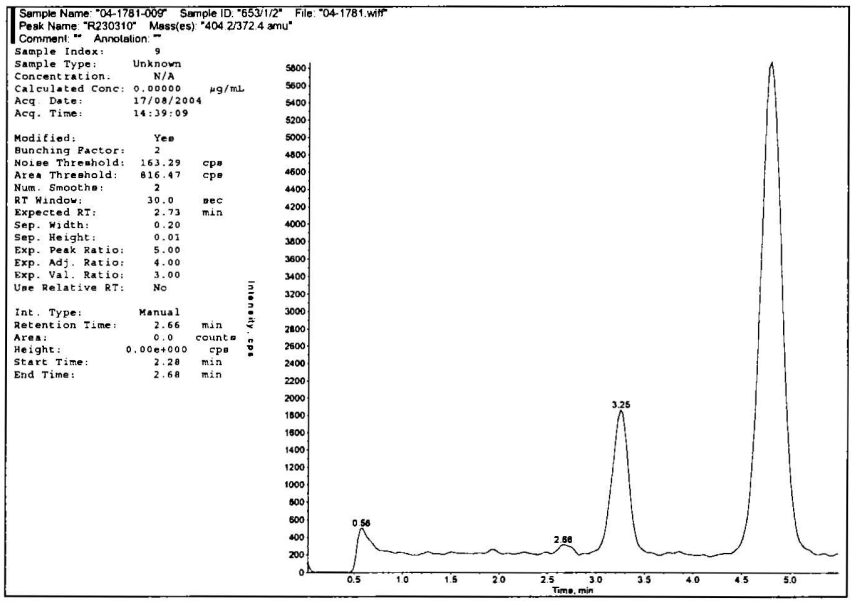


Figure 50: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Sample 04-S616 9/0. R230310 (confirmatory transition) residue = <0.01 mg kg^{-1}.

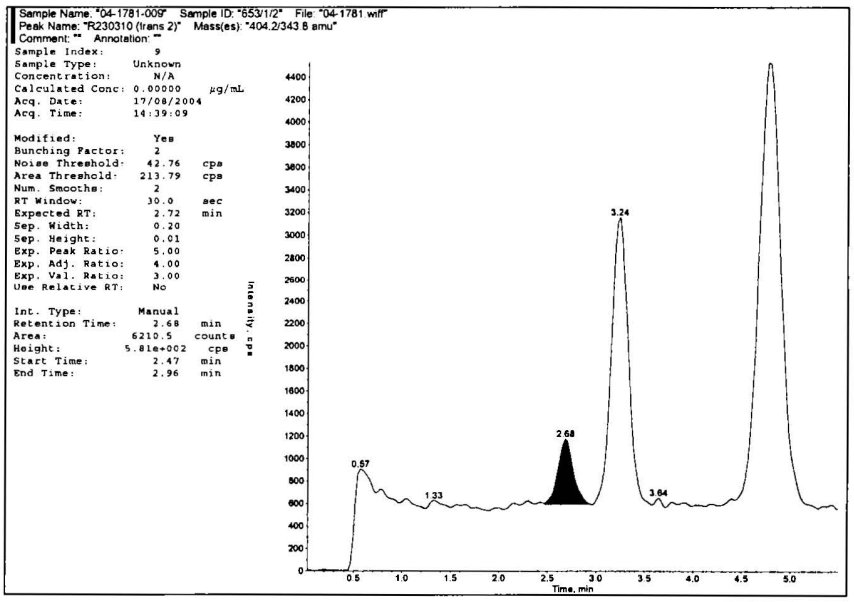


Figure 51: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 100%

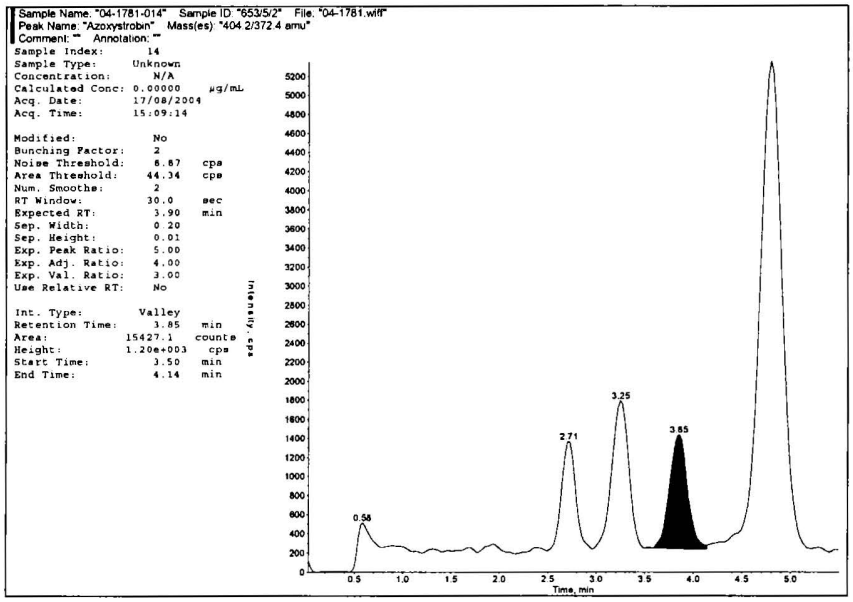


Figure 52: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 101%

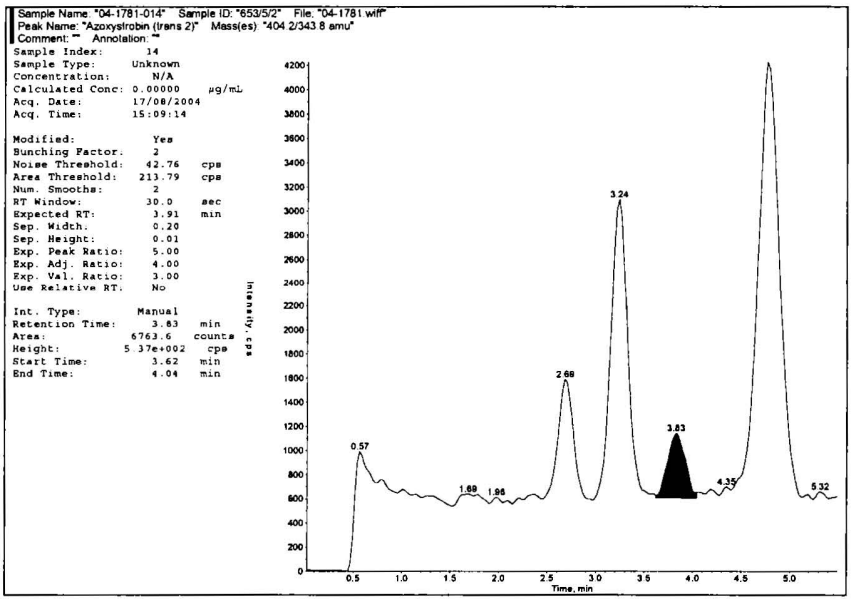


Figure 53: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. R230310 (primary transition) recovery = 96%

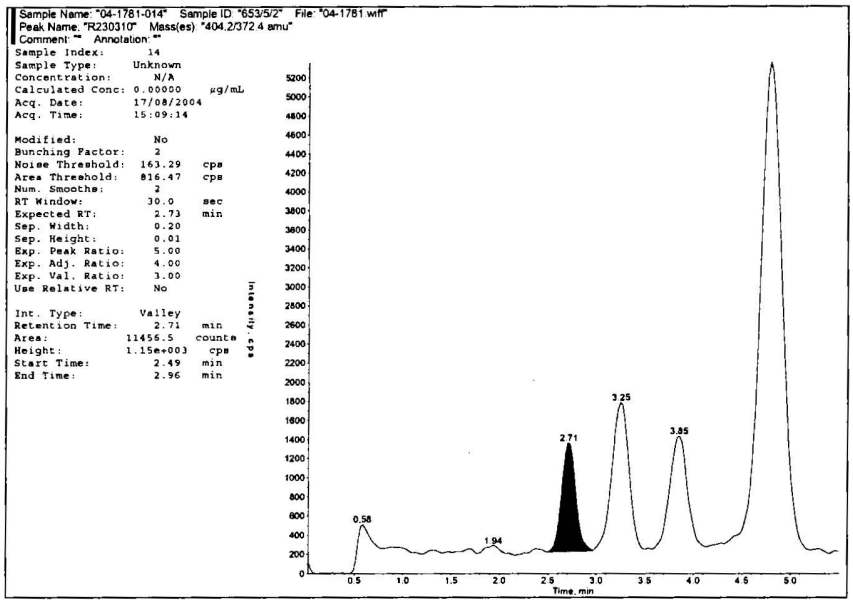


Figure 54: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 0.01 mg kg^{-1}. R230310 (confirmatory transition) recovery = 95%

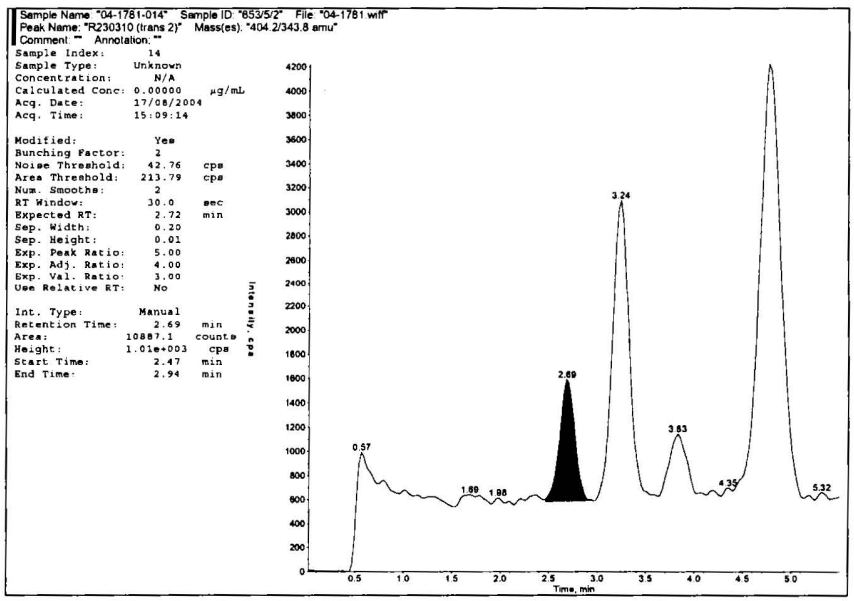


Figure 55: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 10 mg kg^{-1}. Azoxystrobin (primary transition) recovery = 98%

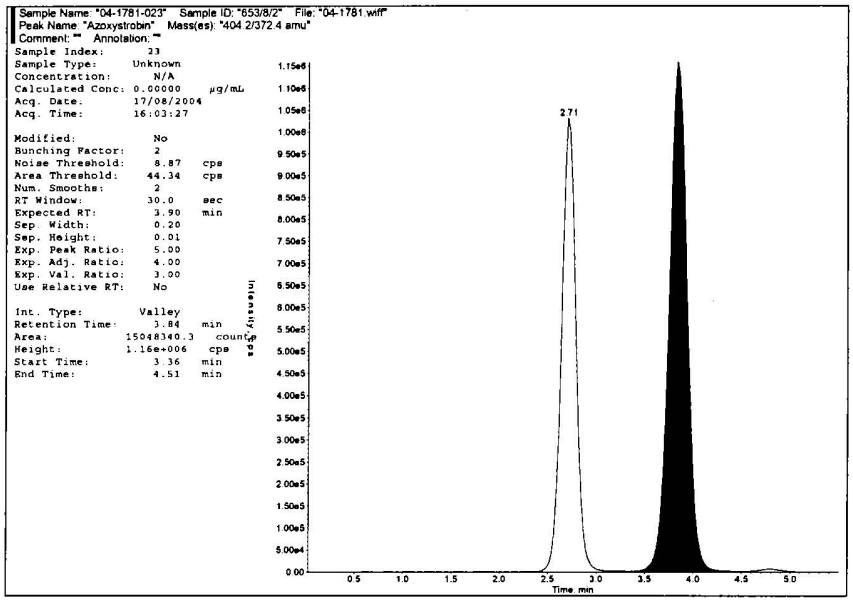


Figure 56: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 10 mg kg^{-1}. Azoxystrobin (confirmatory transition) recovery = 97%

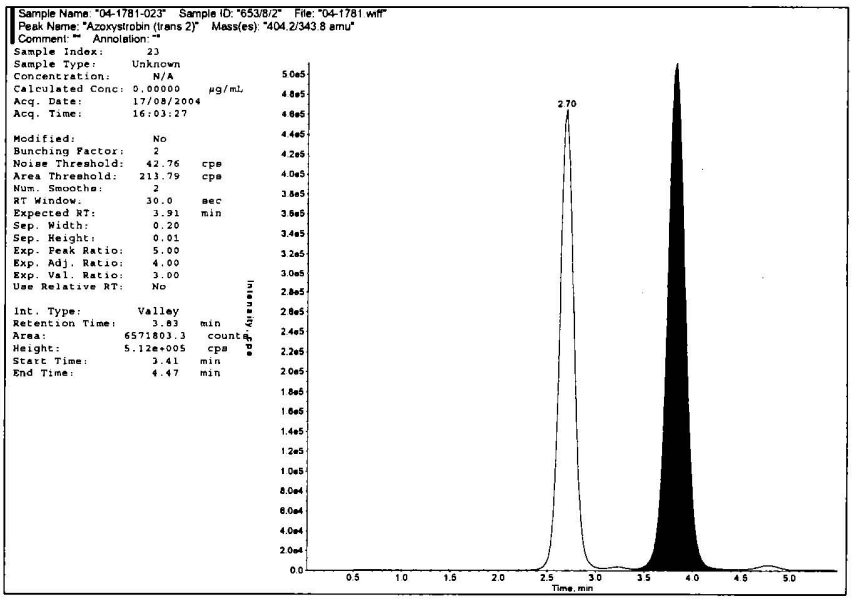


Figure 57: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 10 mg kg^{-1}. R230310 (primary transition) recovery = 92%

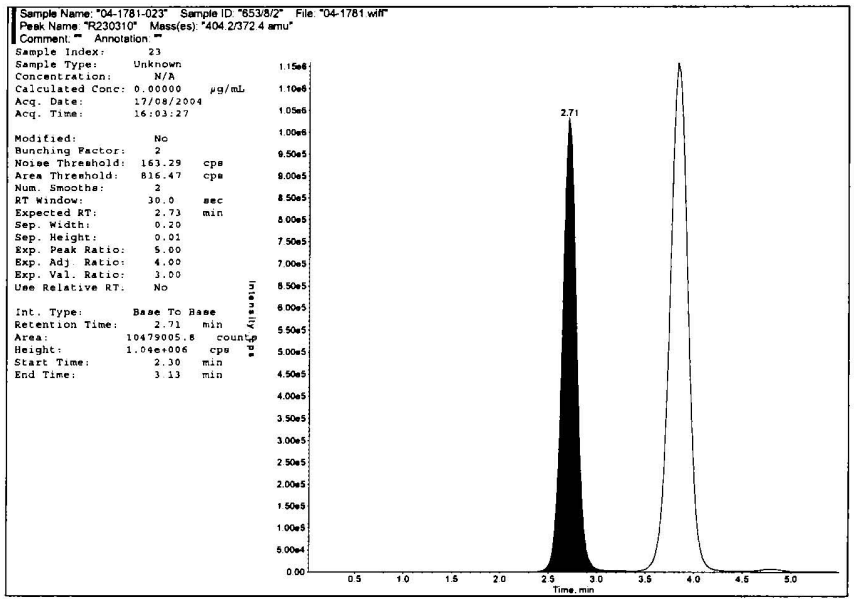
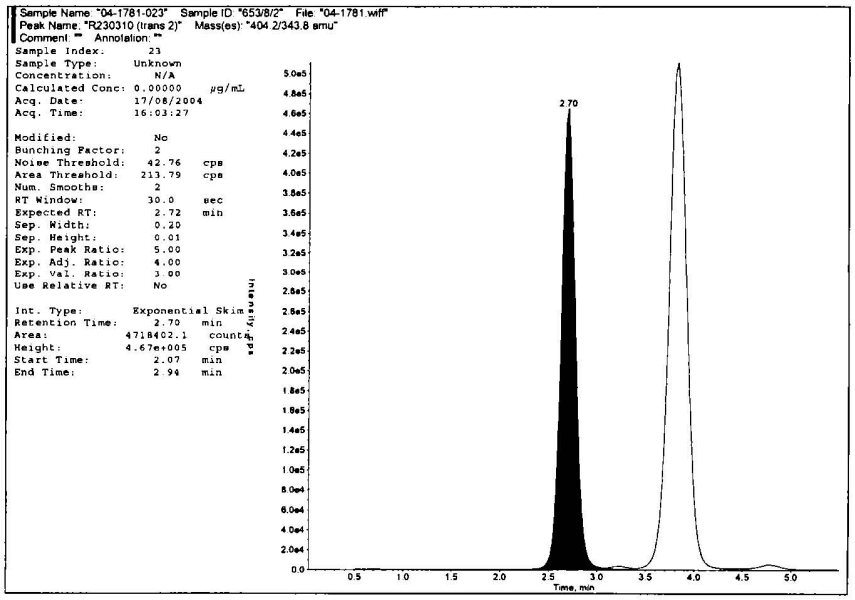


Figure 58: Untreated Sample of Mandarin at 0.01 g mL^{-1}. Fortified at 10 mg kg^{-1}. R230310 (confirmatory transition) recovery = 93%



Appendix 5 : **Detector Linearity Graphs**

Figure 59 : **LC-MS/MS Linearity of Response for Azoxystrobin Primary Transition, *m/z* 404.2 → 372.4, no intercept set. (Ultra-pure Water:Acetonitrile, 50:50, v/v).**

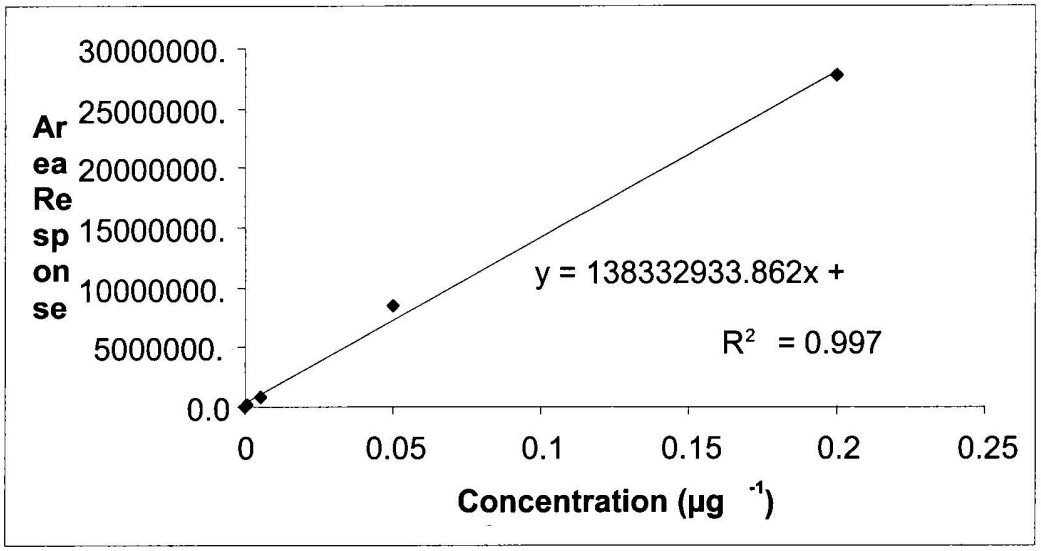


Figure 60 : **LC-MS/MS Linearity of Response for Azoxystrobin Confirmatory Transition, *m/z* 404.2 → 343.8, no intercept set.**
(Ultra-pure Water:Acetonitrile, 50:50, v/v).

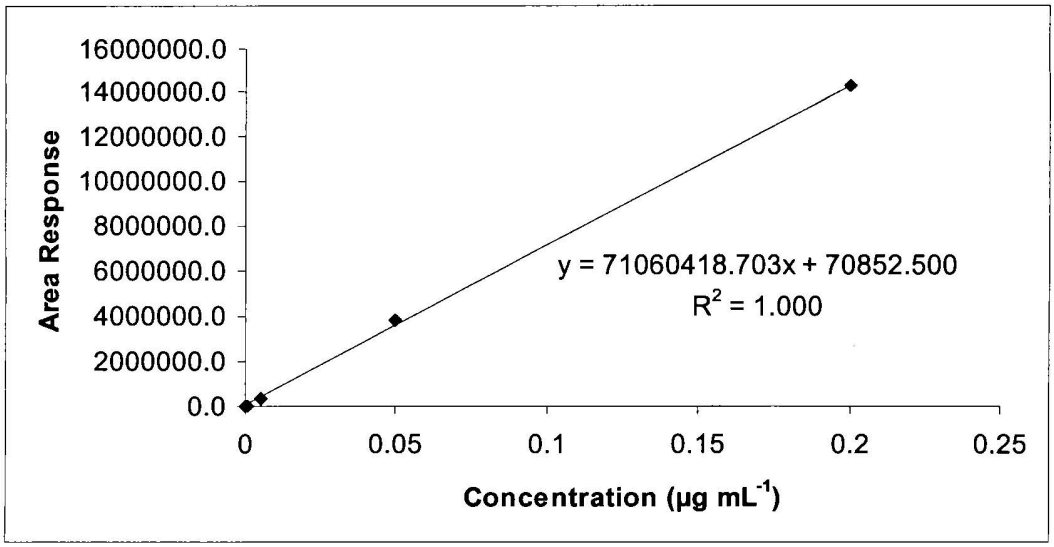


Figure 61 : **LC-MS/MS Linearity of Response for R230310 Primary Transition, *m/z* 404.2 → 372.4, no intercept set. (Ultra-pure Water:Acetonitrile, 50:50, v/v).**

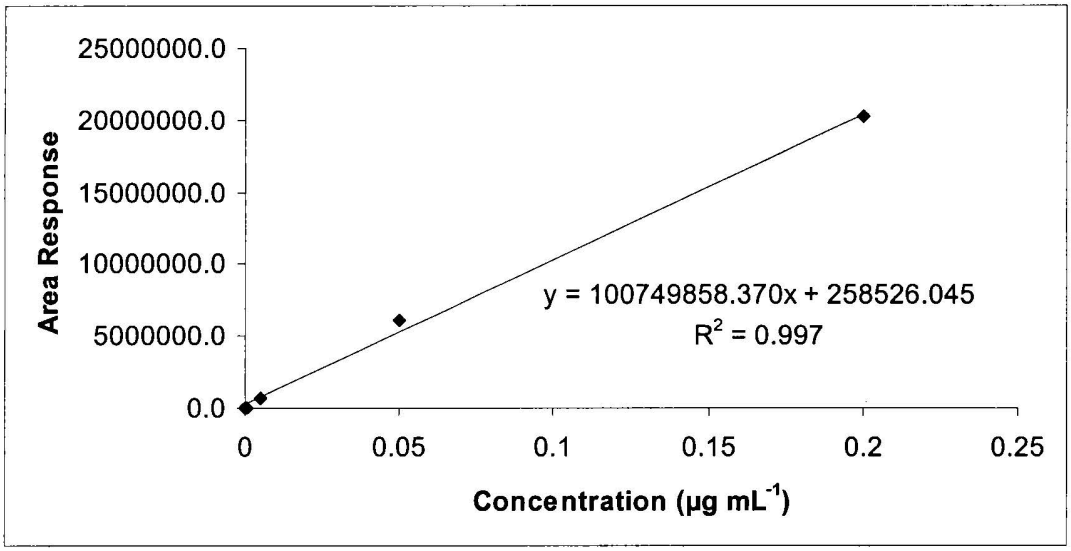


Figure 62 : **LC-MS/MS Linearity of Response for R230310 Confirmatory Transition, *m/z* 404.2 → 343.8, no intercept set. (Ultra-pure Water:Acetonitrile, 50:50, v/v).**

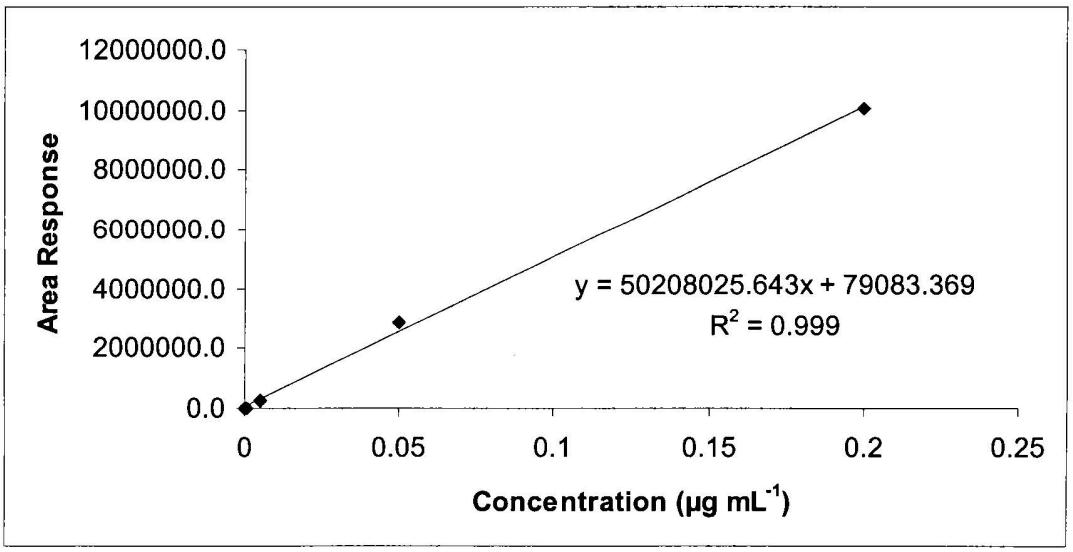


Figure 63 : **LC-MS/MS Linearity of Response for Azoxystrobin Primary Transition, *m/z* 404.2 → 372.4, no intercept set. (Ultra-pure Water:Methanol, 50:50, v/v).**

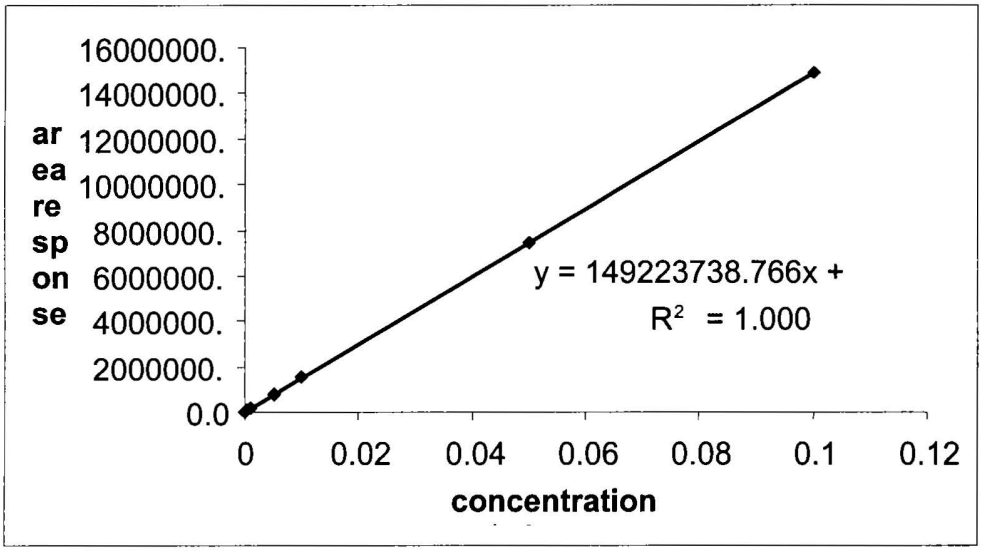


Figure 64 : **LC-MS/MS Linearity of Response for Azoxystrobin Confirmatory Transition, *m/z* 404.2 → 343.8, no intercept set. (Ultra-pure Water:Methanol, 50:50, v/v).**

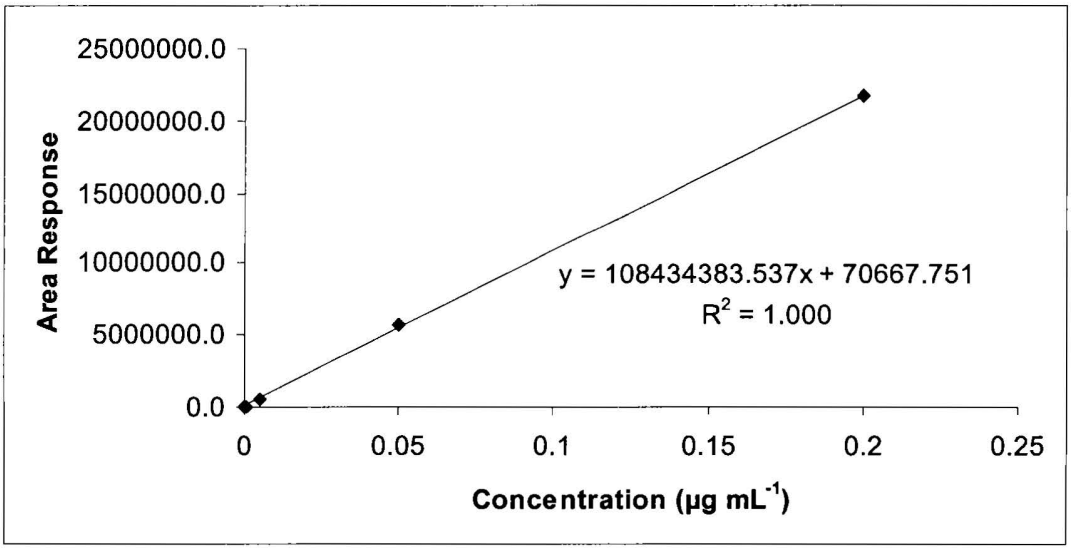


Figure 65: **LC-MS/MS Linearity of Response for R230310 Primary Transition, *m/z* 404.2 → 372.4, no intercept set. (Ultra-pure Water:Methanol, 50:50, v/v).**

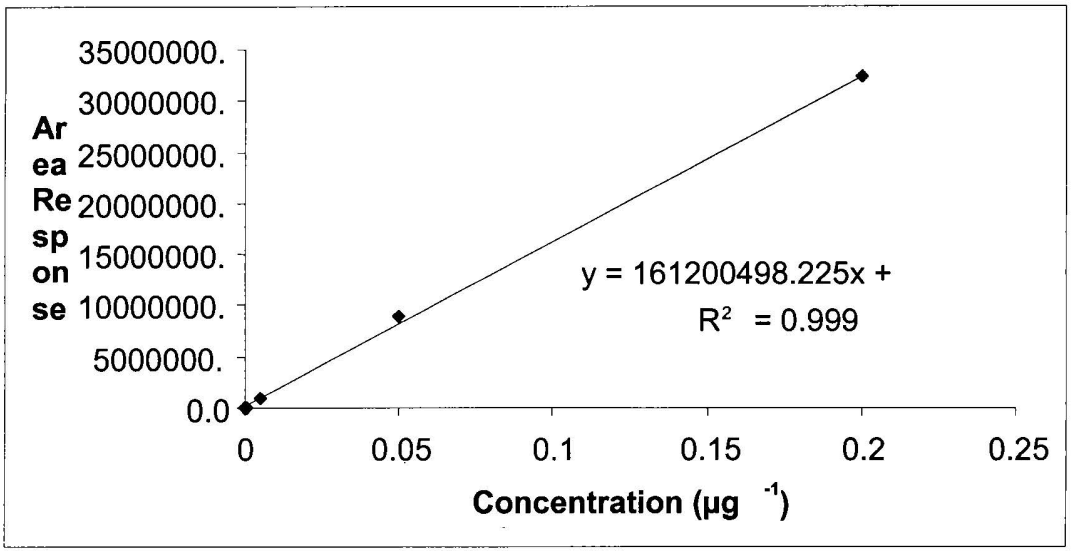
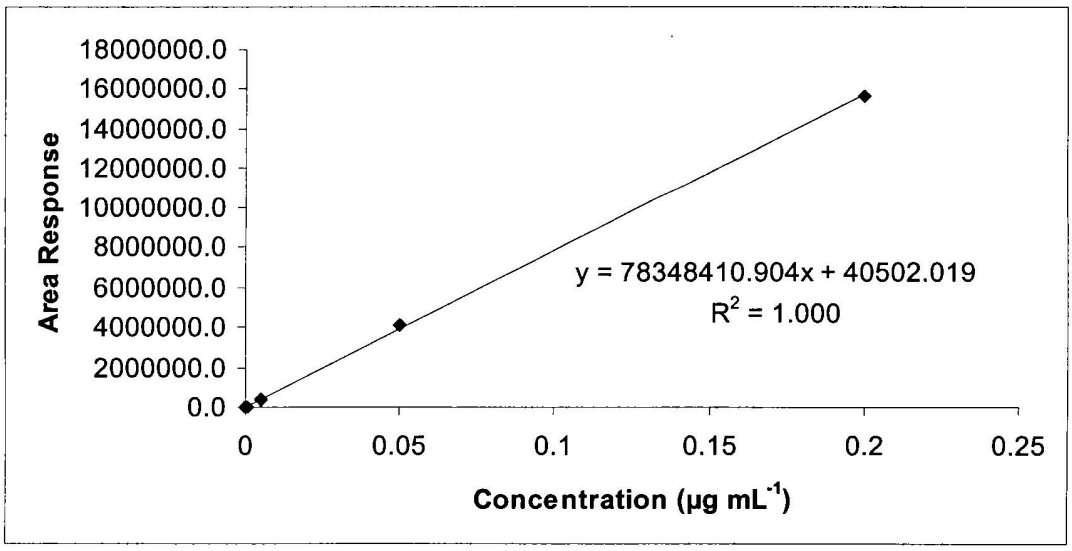


Figure 66 : **LC-MS/MS Linearity of Response for R230310 Confirmatory Transition, *m/z* 404.2 → 343.8, no intercept set. (Ultra-pure Water:Methanol, 50:50, v/v).**



Appendix 6 : API 3000 MS/MS Tuning Procedure

Calibration of Instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufactures instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning of API 3000 MS/MS Instrument for AZOXYSTROBIN

Infuse a standard solution of azoxystrobin (0.1 to 1.0 μg mL^{-1}) in acetonitrile:ultra pure water:acetic acid 50:50:0.2 v/v/v directly into the mass spectrometer interface at a rate at of 5 – 20 μL min^{-1}. Roughly adjust the interface parameters (sprayer position, spray, heater and auxiliary gas flows, in addition to spray, orifice, and focusing ring voltages) for a sufficiently high parent ion signal at *m/z* 404.

Using the Analyst software quantitative optimisation programme, tune the instrument for azoxystrobin, ensuring that the correct ions are selected (initial Q1 *m/z* = 404 and product ions *m/z* = 372 and 344). If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of azoxystrobin standards using a mobile phase of 50:50 (v/v) ultra pure water:acetonitrile + 0.2 % acetic acid at the required flow rate and at the intended split ratio. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.

Figure 67 : Azoxystrobin Initial Product Scan (positive ionisation)

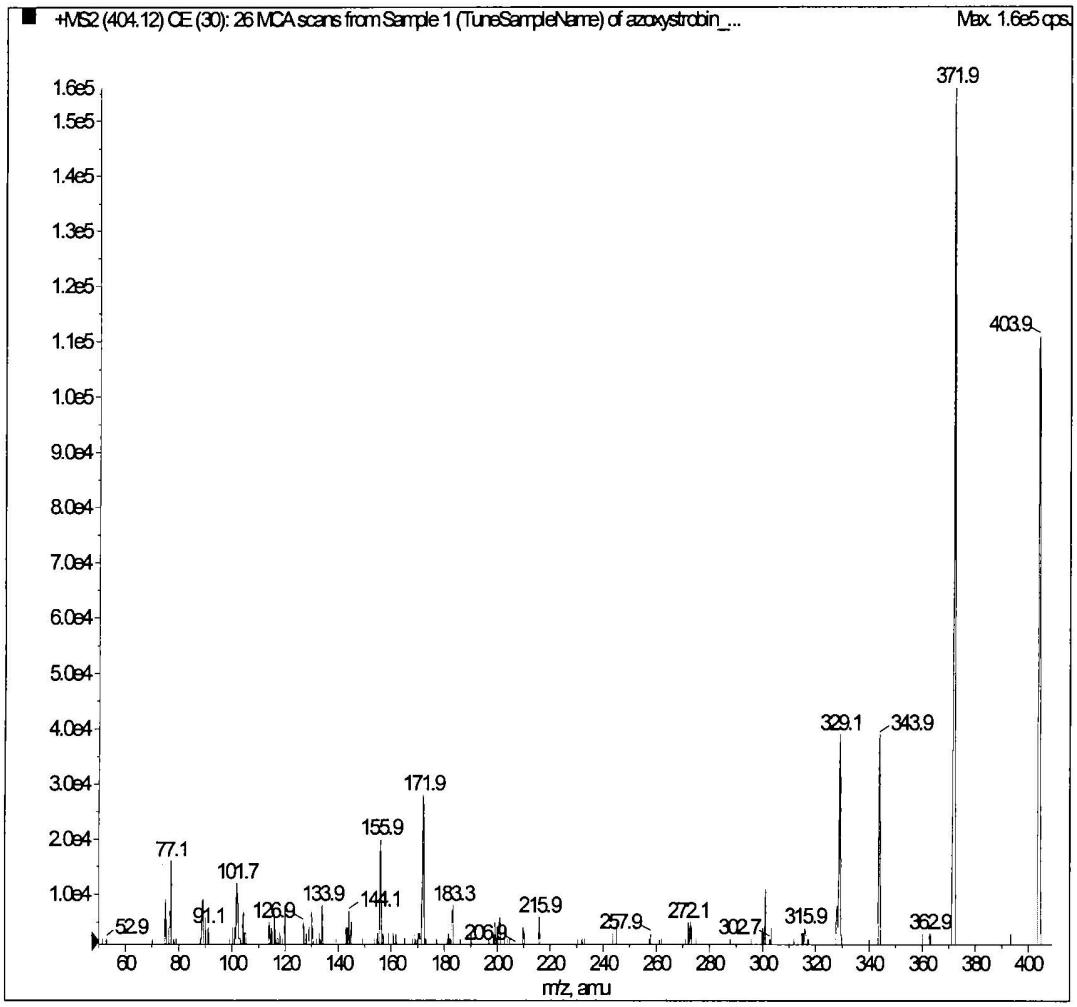


Figure 68 : Azoxystrobin Final Product Scan (positive ionisation)