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REM 130.11

**RESIDUE ANALYTICAL METHOD FOR THE
DETERMINATION OF RESIDUES OF PROPICONAZOLE (CGA 64250) IN CROP SAMPLES. FINAL
DETERMINATION BY LC-LC-MS/MS.**

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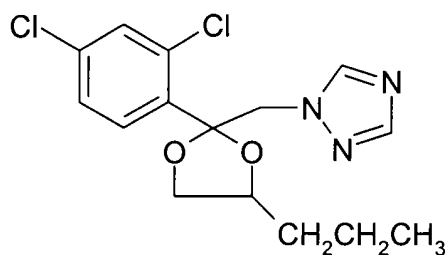
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1. Introduction and Summary

1.1 Scope

The analytical procedure described is suitable for the determination of residues of propiconazole (Figure 1) in crop samples using an external standardisation procedure. The limit of quantification (LOQ) has been set at 0.01 mg kg⁻¹.

Figure 1 : Propiconazole
IUPAC Name : 1-{2-(2',4'-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl}-1H-1,2,4-triazole
Molecular Mass : 342.22



1.2 Method Summary

Samples are extracted by shaking with methanol: water (80:20 v/v). Extracts are filtered and aliquots are diluted with methanol: ultra-pure water (5:95 v/v). Final determination is by high performance liquid chromatography using a two-column switch with triple quadrupole mass spectrometric detection (LC-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/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 propiconazole analytical standard to allow dilution in ethanol to give a $200 \mu\text{g mL}^{-1}$ stock solution in a volumetric flask. This standard should then be diluted by serial dilution to $0.1 \mu\text{g mL}^{-1}$ in methanol. A further set of dilutions should be prepared to $0.0001 \mu\text{g mL}^{-1}$ in methanol: ultra pure water (20:80 v/v). These should be used as calibration standards for final determination by LC-LC-MS/MS.

When not in use, always store the standard solutions in a refrigerator at $\leq 7^{\circ}\text{C}$ to prevent decomposition and/or concentration of the standard. 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

	Ethanol	Methanol	Acetonitrile	Formic Acid
Harmful Vapour	✓	✓	✓	✓
Highly Flammable	✓	✓	✓	✗
Harmful by Skin Absorption	✓	✓	✓	✓
Syngenta Divisional Toxicity Class	3	3	3	3
OES Short Term (mg m ⁻³)		310	105	N/A
OES Long Term (mg m ⁻³)	1900	260	70	5

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

At present there is insufficient data available to assign a Syngenta Toxicity Classification for propiconazole. It should be treated as a class 3 compound until further information indicates otherwise.

The toxicity classification scale rates highly toxic chemicals as class 1 and non-toxic chemicals as class 5.

2.5 Time Required for Analysis

The methodology is normally performed with a batch of up to 12 samples. One person can complete the analysis of up to 12 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 $\leq 7^{\circ}\text{C}$.

2.7 Modifications and Potential Problems

- Sample extracts should preferably be analysed by LC-LC-MS/MS on the same day that they are prepared.
- 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.

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) into plastic centrifuge bottles (250 mL size). At least one untreated control and two control samples fortified with known amounts of propiconazole in methanol (not more than 0.5 mL) should be analysed with each batch of samples using the same procedure to enable verification of the method and recovery corrections to be made.

Add methanol: ultra pure water 80:20 v/v (100 mL minus the water content of the samples). Mechanically shake the centrifuge bottle for two hours. (100-150 rpm is sufficient to give homogenous extracts).

Note: Use the composition of foods handbook (Reference 3) to 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 relevant information can 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 above sources, it may be necessary to determine the moisture content following a suitable moisture content determination procedure e.g. SOP ESJH/309/--.(Reference 4)

- b) Filter the sample under gravity through filter paper such as Whatman Grade 1 until there is sufficient filtered extract to measure 2.0 ml aliquots accurately.

The sample concentration is now 0.1 g mL⁻¹.

3.3 Sample Dilution

- a) Transfer aliquots of the filtered crop extracts, equivalent to 0.2 g (2.0 mL) into appropriate vessels (e.g. 15mL screw capped, graduated, plastic tubes). Add methanol: ultra pure water 5:95 v/v (8 mL) to give a final volume of 10 mL.
- b) Ultrasonicate thoroughly and transfer samples to a suitable autosampler vial for analysis by LC-LC-MS/MS. The sample concentration is now 0.02 g mL⁻¹.

3.4 LC-LC-MS/MS Calibration Standards

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

Suppression or enhancement of the LC-LC-MS/MS response to propiconazole in the presence of matrix was 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 is observed a matrix-matched standard may be used to compensate at the discretion of the study director.

- c) For example, to prepare a 0.0002 µg mL⁻¹ propiconazole matrix matched standard, take a further 0.2 g control aliquot at section 3.3 (a). Add 20µL of 0.1 µg mL⁻¹ propiconazole standard in methanol to 1.98 mL filtered control extract, then add methanol: ultra pure water 5:95 v/v (8.0 mL) to give a final volume of 10 mL in 20:80 v/v methanol: ultra pure water. Ultrasonicate thoroughly and transfer to a suitable autosampler vial. The concentration of the matrix matched calibration standard is now 0.0002 µg mL⁻¹.

4. Final Determination by LC-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 4000 triple quadrupole mass spectrometer
Auto sampler	: CTCPAL
Gas Supply	: Peak Scientific NM20ZA gas station

4.2 Chromatography Conditions

Column 1	: Inertsil Phenyl, 5 μm , 250 mm x 2.1 mm I.D.
Column 2	Inertsil Phenyl, 3 μm , 100 mm x 2.1 mm I.D.
Flow rate	: 250 $\mu\text{L min}^{-1}$
Injection volume	: 30 μL
Injection protocol	: Analyse calibration standard after 3 to 4 sample injections
Typical Retention Times	: Column 1: 8.0 min (cut:7.2 - 8.3 min); Column 1+2: 10.1 min
Mobile phases	: 1: acetonitrile:ultra pure water (55:45 v/v) + 0.1% formic acid 2: acetonitrile:ultra pure water (75:25 v/v) + 0.1% formic acid

4.3 Determination of switching interval and typical values

Determine actual switching times either each time a series of samples is to be analysed or, if the system performance is sufficiently stable over a longer period, if a relevant loss of sensitivity is detected. For the determination of the switching time connect the outlet of the switching valve (after column 1) or the outlet of column 1 to the MS/MS detector. Inject a standard solution of propiconazole (eg. $0.01\mu\text{g mL}^{-1}$) in methanol: ultra pure water 20:80 v/v. Determine start time and end time of the switching interval in order to transfer the complete peak. If necessary, take into account dead times induced by the connection tubes.

Typical retention times for propiconazole are:

-Column 1: 8.0 min (cut 7.2-8.3 min)

-Column 1+2: 10.1 min

4.4 API 4000 Mass Spectrometer Conditions

Interface	:	TurboIonSpray
Polarity	:	Positive
Nebuliser gas 1 (GS1)	:	60 (arbitrary units)
Nebuliser gas 2 (GS2)	:	60 (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 7 (arbitrary units)
Scan type	:	MRM

			Propiconazole
Q1 m/z	:		343.13
Q3 m/z	:		158.95
Dwell time	:		300 ms
Resolution Q1	:		Unit
Resolution Q3	:		Unit
Declustering potential (DP)	:		66 V
Entrance potential (EP)	:		10 V
Collision energy (CE)	:		49 V

Collision cell exit potential (CXP)	:	10 V
Electron multiplier setting (CEM)	:	1800 V

Protonated molecular ions generated in the ion source (propiconazole m/z 343) are selected and subjected to further fragmentation by collisional activation. The most abundant ions (m/z 159) in the resulting daughter spectra are then monitored and used for quantitative analysis. No confirmatory conditions are included as final determination by LC-LC-MS/MS is considered to be highly specific. Typical chromatograms are shown in Appendix 4. Initial and final product scans showing the fragmentation and daughter ions for propiconazole are presented in Appendix 6.

5. Calculation of Results

Residues may be calculated using an external standardisation procedure.

Propiconazole 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 propiconazole at an appropriate concentration into the LC-LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak area obtained for propiconazole.
- b) Make an injection of each sample solution and measure the peak heights or areas of the peaks corresponding to propiconazole.
- c) Re-inject the standard solution after a maximum of four injections of sample solutions.
- d) Calculate the propiconazole 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 ($\mu\text{g mL}^{-1}$)

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

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

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

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

6. Control and Recovery Experiments

The levels of external recoveries should be decided by 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 propiconazole prior to extraction).

Control and external recovery experiments should be completed as section 3 for each set of samples analysed. Provided the recovery values are acceptable they may be used to correct any propiconazole residues found.

Recovery data is generally considered acceptable when the mean values are between 70% and 110% and with a coefficient of variation of $\leq 20\%$.

7. Specificity

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

7.1 Matrix

LC-LC-MS/MS is a highly specific detection technique. Significant interference arising from the crop matrices tested has not been observed.

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. $\geq 0.01 \mu\text{g mL}^{-1}$) 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 Recoveries

A method validation study demonstrating acceptable recovery data and repeatability has been carried out on the procedures described in Section 3. This is reported in RJ3496B (Reference 5). 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 c.v. of $\leq 20\%$ 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 LOQ has been set at 0.01 mg kg^{-1} for LC-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 0.002 mg kg⁻¹.

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 API4000 MS/MS detector response for propiconazole standards prepared in methanol: ultra pure water 20:80 (v/v) was tested in the range from 0.0001 to 0.004 µg mL⁻¹ concentration (equivalent to 3 - 120 pg injected on column when using a 30 µL injection volume) and was found to be linear.

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. A t-value of 1.19 for propiconazole was obtained with 3 degrees of freedom. The tabular t value at the 10% level of significance, with 3 degrees of freedom, is 2.35. Since the computed t value is smaller than the tabular t value, at the 10% 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 6).

If residues beyond the tested concentration range are expected, dilute the extract appropriately to bring it within the tested linear range prior to quantification.

8.4 Limitations

The method has been tested on barley grain, barley straw, barley forage, apple and oil seed rape matrices. 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 propiconazole residues in crops. Only commercially available laboratory equipment and reagents are required. One person can complete the analysis of a batch of up to 12 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 LC-LC 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. Watt B K and Merrill 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.
4. Syngenta Standard Operating Procedure SOP ESJH/309/--: Determination of moisture contents.
5. Ely S V (2004): Propiconazole: Validation of a Residue Analytical Method REM 130.11 for the Determination of Residues in Crops. Syngenta report number RJ3496B.
6. 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.

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Reference : SJRv2.0
PIT no. : F064250GBL002A-1221
Date : 14 December 2004

Appendices

Appendix 1 : Apparatus

UK Suppliers

Mechanical shaker for extraction of samples available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

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.

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

Nalgene™ polypropylene centrifuge tubes, 15 mL capacity with 0.1 mL graduations. Available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number CFT-430M.

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.

API4000 LC-MS-MS system equipped with a TurboIonSpray source, available from Applied Biosystems, Kelvin Close, Birchwood Scientific Park North, Warrington, Cheshire WA3 7PB, UK.

HPLC columns, Intersil Phenyl 5µm, 250mm x 2.1mm, and Inertsil Phenyl 3µm, 100mm x 2.1mm available from Phenomenex, Queens Avenue, Hurdsfield Ind Estate, Macclesfield, Cheshire, SK10 2BN

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 9REUS Suppliers

Mechanical shaker for extraction of samples available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

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.

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

Nalgene™ polypropylene centrifuge tubes, 15 mL capacity with 0.1 mL graduations. Available from Nalge Company, 75 Panorama Creek Drive, PO Box 20365, Rochester, NY 14602-0365.

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.

API4000 LC-MS-MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128, USA.

HPLC column, Intersil Phenyl 5µm particle size, 250mm x 2.1mm, and Inertsil Phenyl 3µm, 100mm x 2.1mm available from, available from Phenomenex, 2320 W. 205th St. Torrance, CA 90501-1456

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, ethanol, methanol, super purity grade and bottled HPLC grade water, available from Rathburn Chemicals Ltd., Walkerburn, Scotland EH43 6AU, UK.

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

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

US Suppliers

Acetonitrile, ethanol, methanol, 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).

Formic 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.

Appendix 3 : Method Validation Data

Table 1. : Propiconazole Recovery Data Obtained During Method Validation

Matrix	Fortification Level (mg kg ⁻¹)	Recovery (%)	Mean (%)	RSD (%)	Range
Apple	Control	ND			
	0.01*	111,112,109,110,111	111	1	109 - 112
	0.1	109,109,110,109,112	110	1	109 - 112
	Overall		110	1	109 - 112
Winter Barley Grain	Control	ND			
	0.01*	108,112,103,94,106	105	6	94 - 112
	0.1	101,101,104,110,109	105	4	101 - 110
	Overall		105	5	94 - 112
Winter Barley Straw	Control	ND			
	0.01*	100,103,95,100,106	101	4	95 - 106
	0.1	81, 83, 89, 86, 83	84	4	81 - 89
	Overall		93	10	81 - 106
Oil Seed Rape	Control	ND			
	0.01*	99,101,98,105,100	101	3	98 - 105
	0.1	97, 88, 96, 92, 92	93	4	88 - 97
	Overall		97	5	88 - 105
Barley Forage	Control	ND			
	0.01*	92, 88, 84, 93, 94	90	5	84 - 94
	0.1	86, 86, 89, 85, 83	86	3	83 - 89
	Overall		88	5	83 - 94

ND no residues on or near LOQ determined in controls analysed in duplicate.

*Limit of quantification, defined by the lowest validated fortification level

Appendix 4 : Representative Chromatograms

Figure 2 : 0.002 $\mu\text{g mL}^{-1}$ Propiconazole standard.

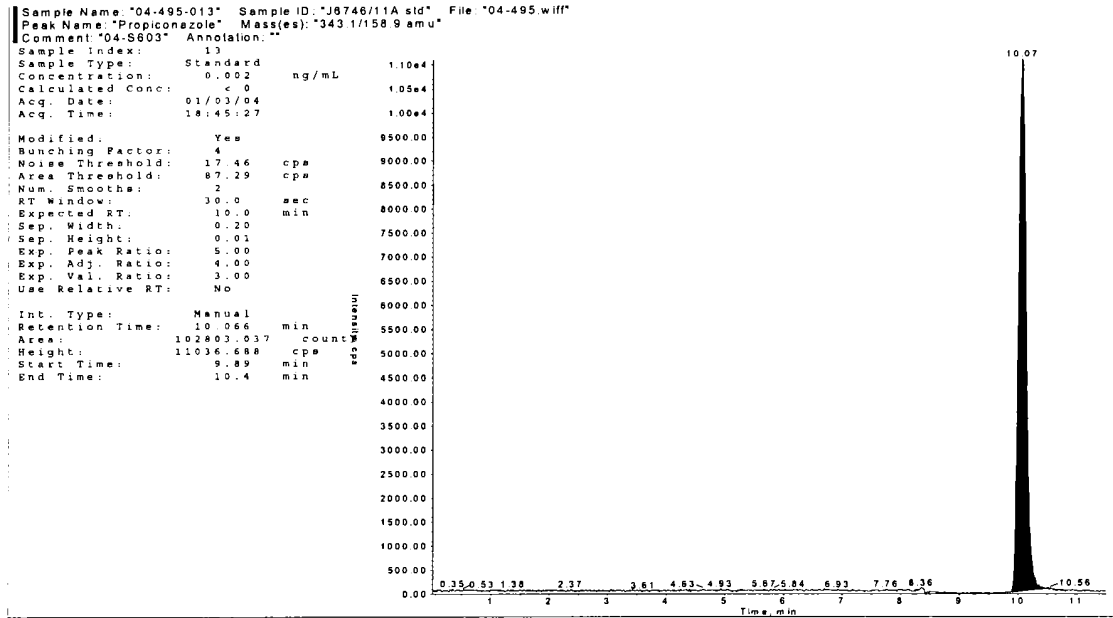
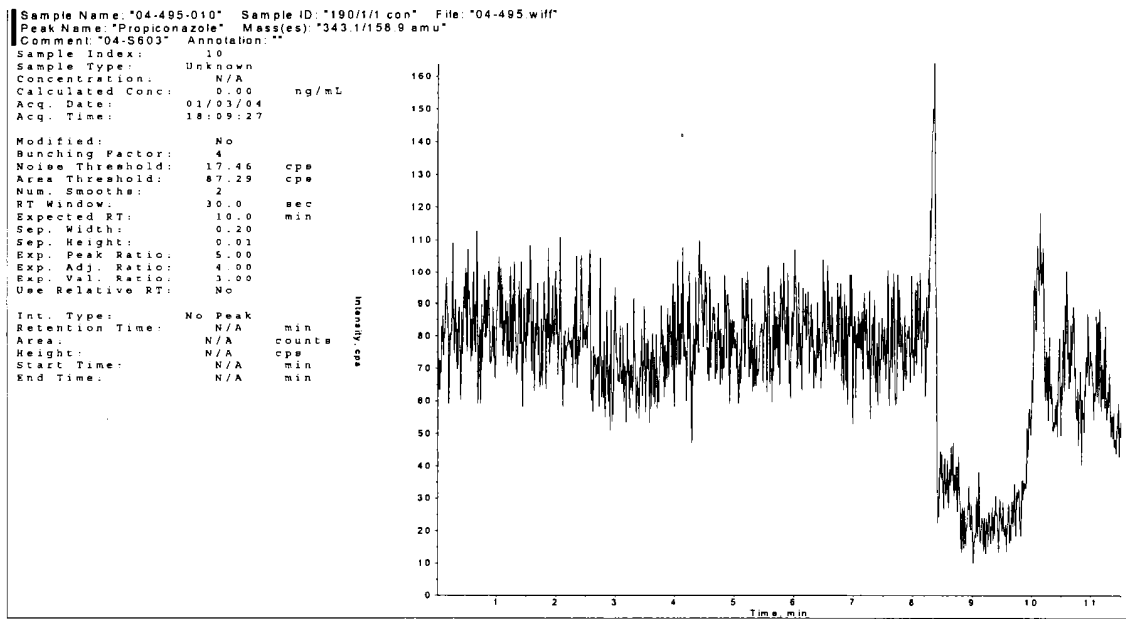
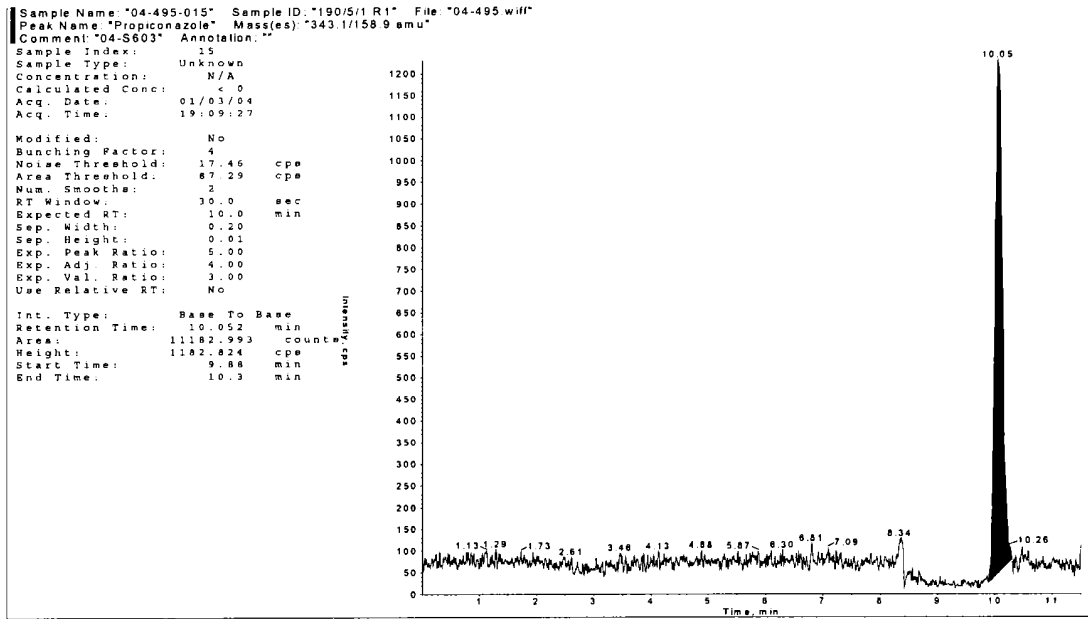


Figure 3 : Untreated apple at 0.02 g mL^{-1} .



**Figure 4 : Untreated apple fortified at 0.01 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 109%.**



**Figure 5 : Untreated apple fortified at 0.1 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 109%.**

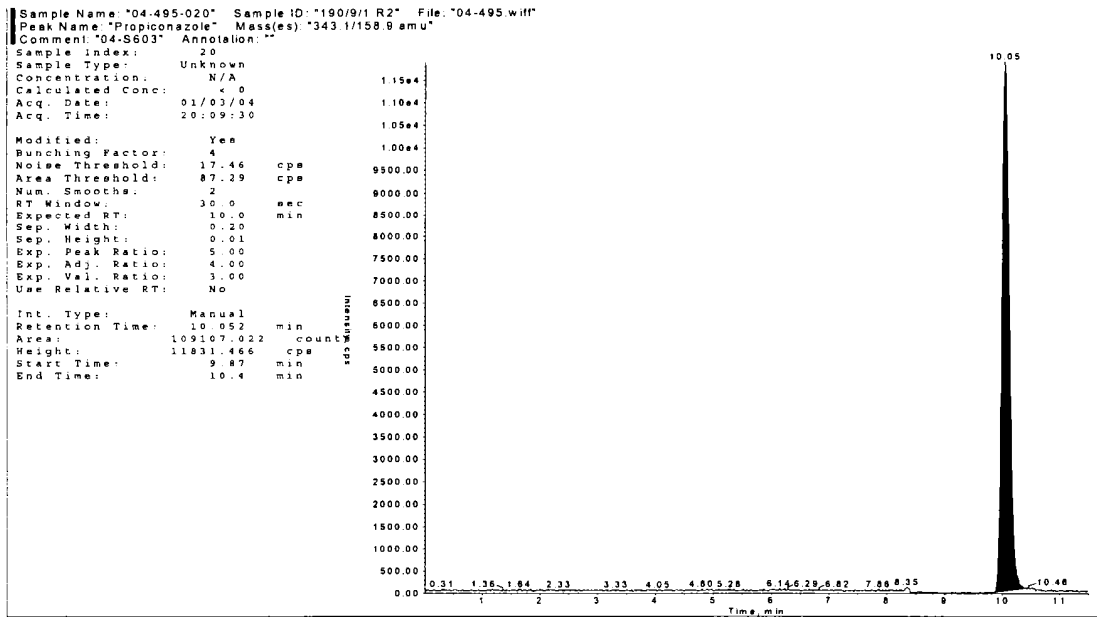


Figure 6 : 0.002 $\mu\text{g mL}^{-1}$ Propiconazole standard.

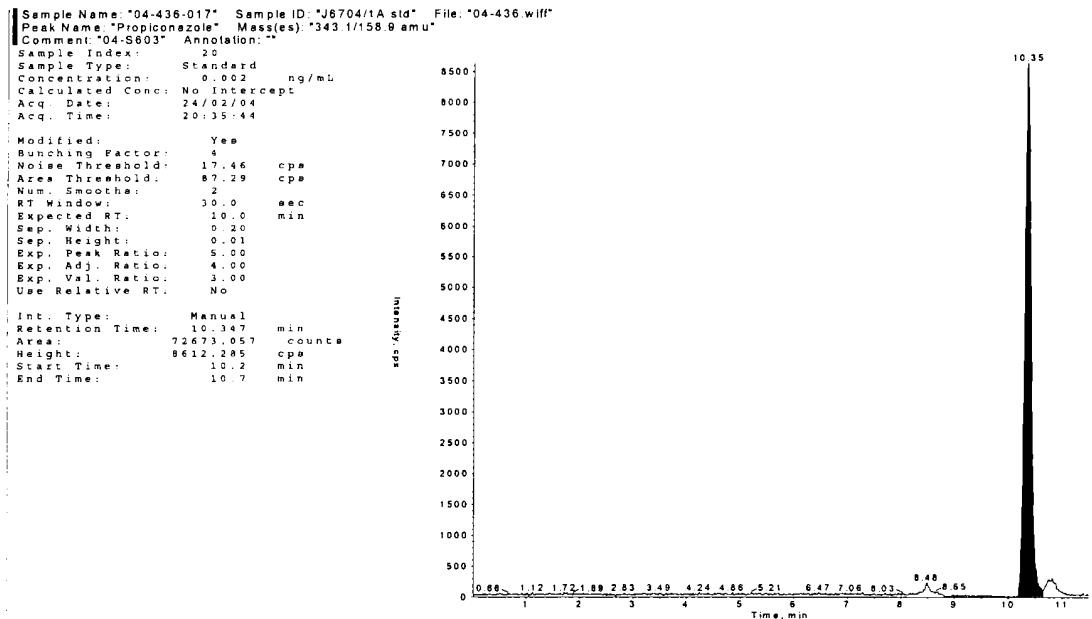
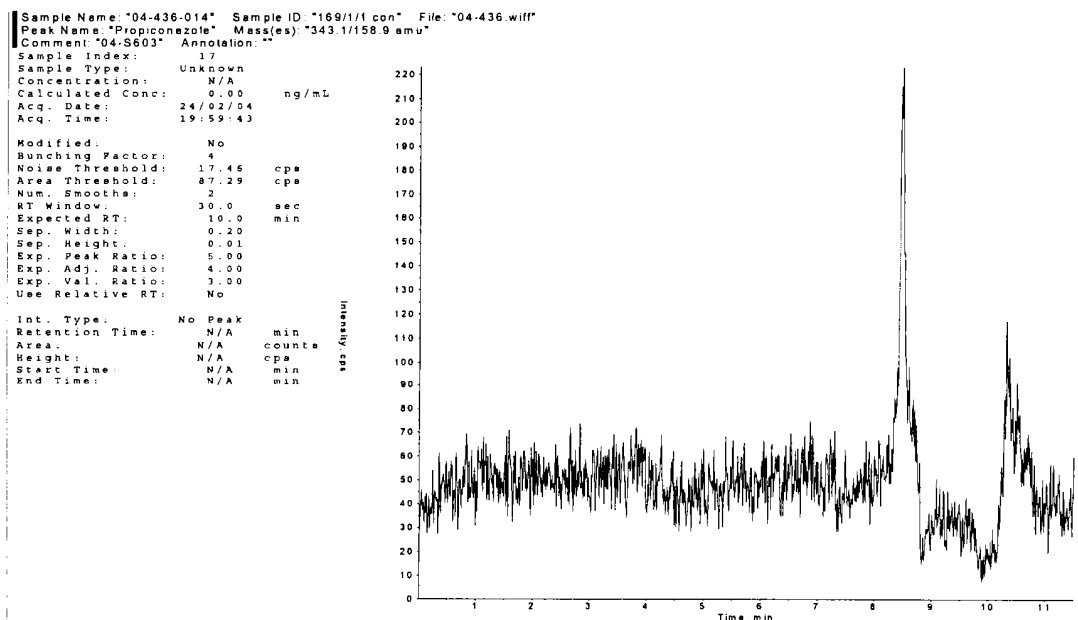
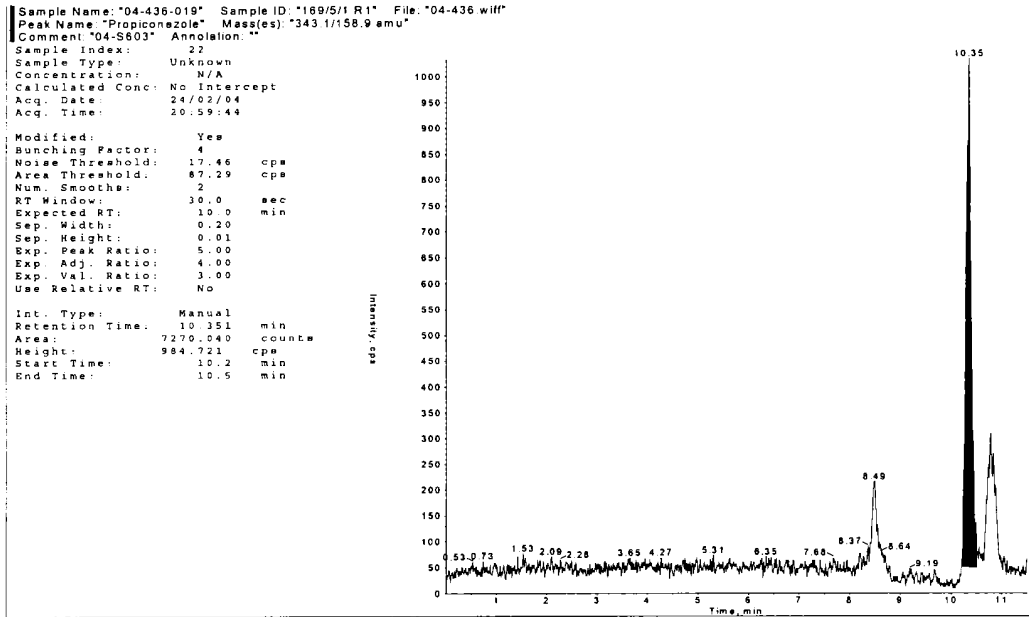


Figure 7 : Untreated winter barley grain at 0.02 g mL^{-1} .



**Figure 8 : Untreated winter barley grain fortified at 0.01 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 103%**



**Figure 9 : Untreated winter barley grain fortified at 0.1 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 101%**

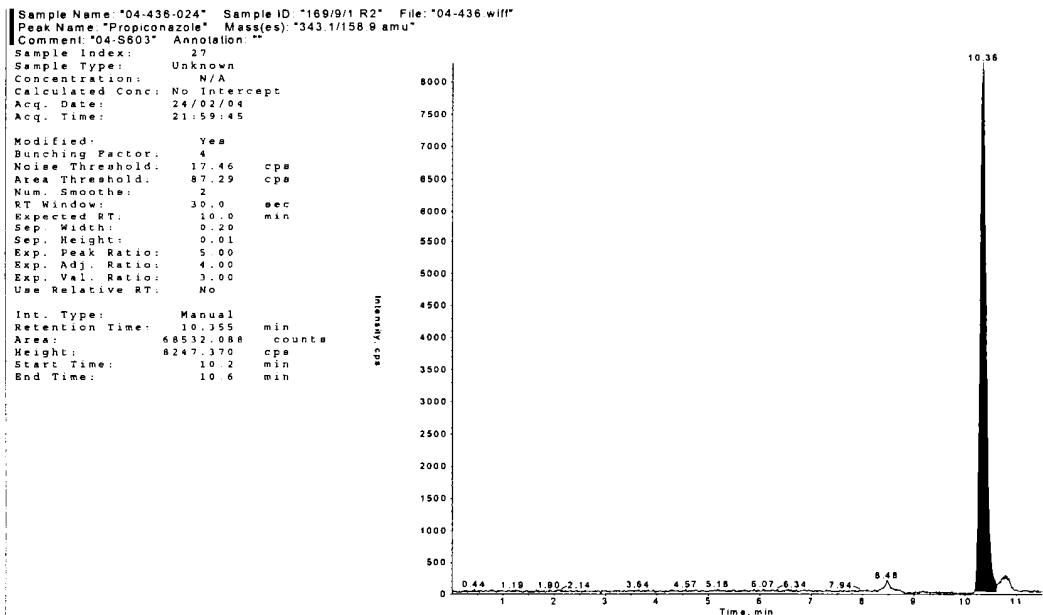


Figure 10 : 0.002 $\mu\text{g mL}^{-1}$ Propiconazole standard.

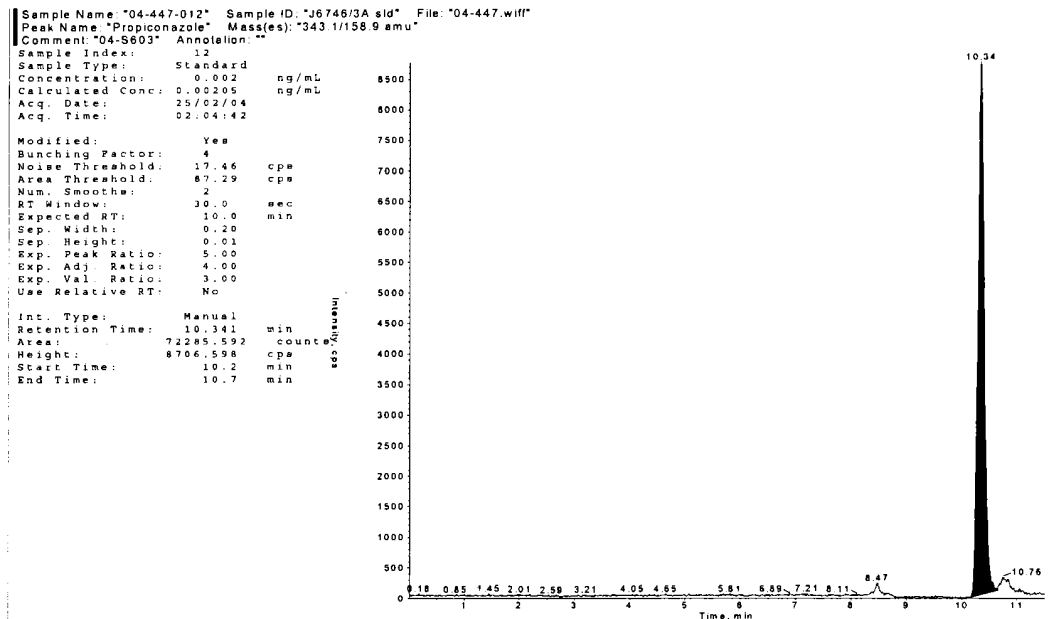
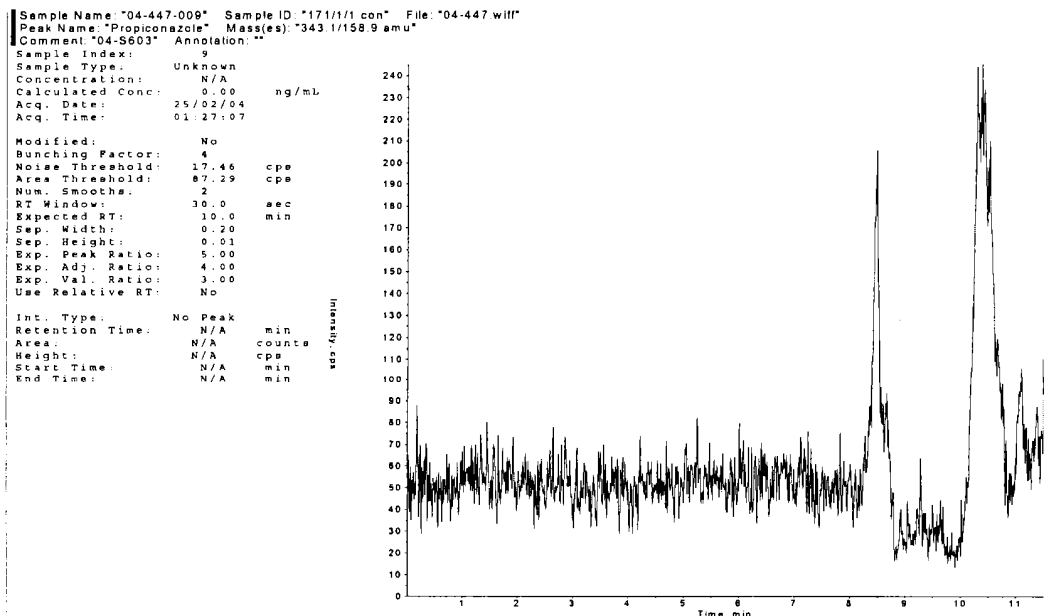
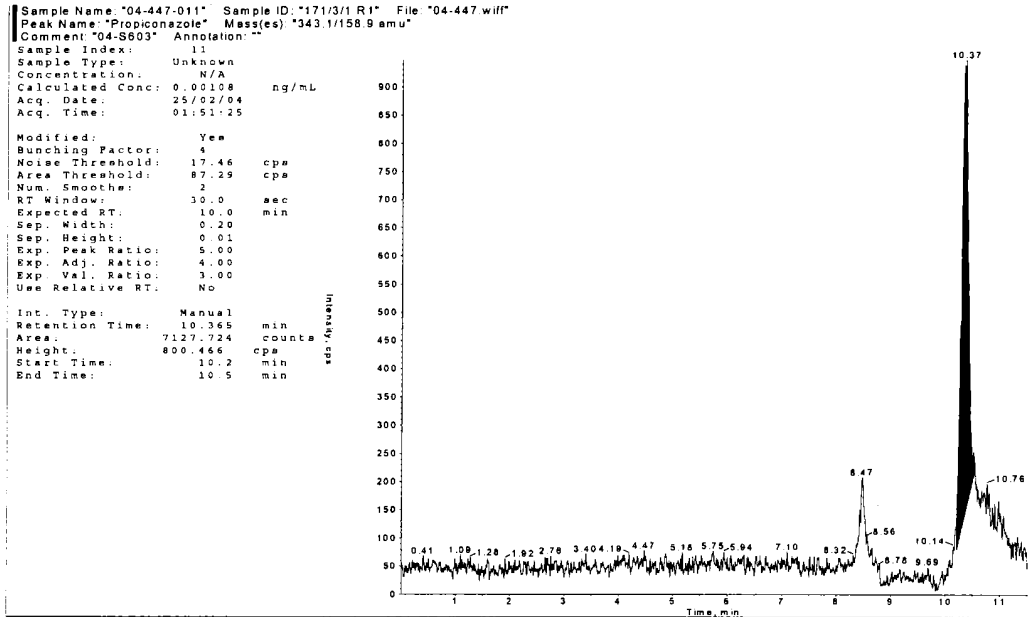


Figure 11 : Untreated winter barley straw at 0.02 g mL^{-1}



**Figure 12 : Untreated winter barley straw fortified at 0.01 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 95%.**



**Figure 13 : Untreated winter barley straw fortified at 0.1 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 89%.**

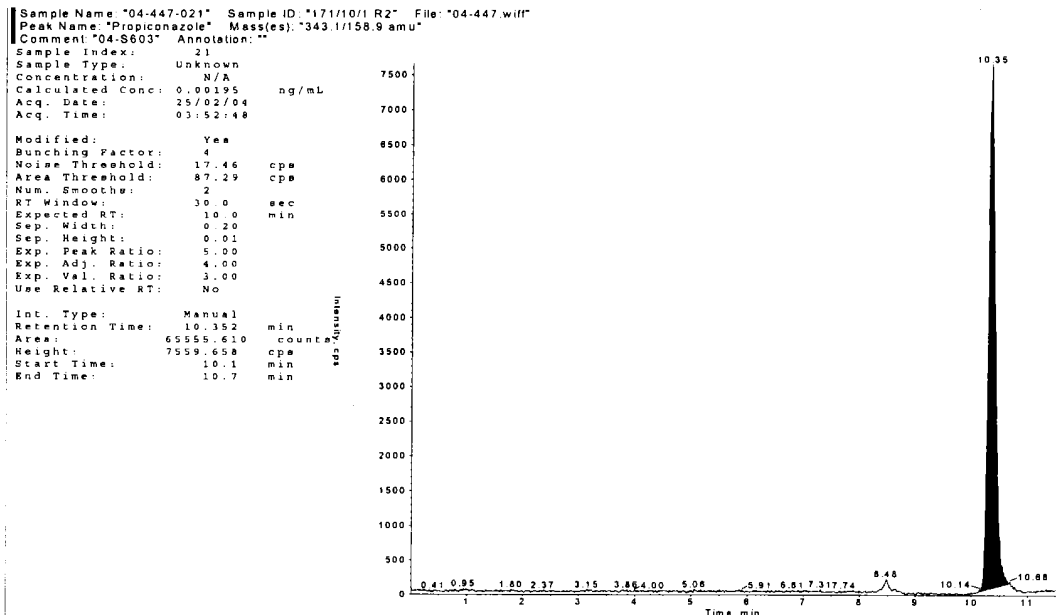


Figure 14 : 0.002 $\mu\text{g mL}^{-1}$ Propiconazole standard.

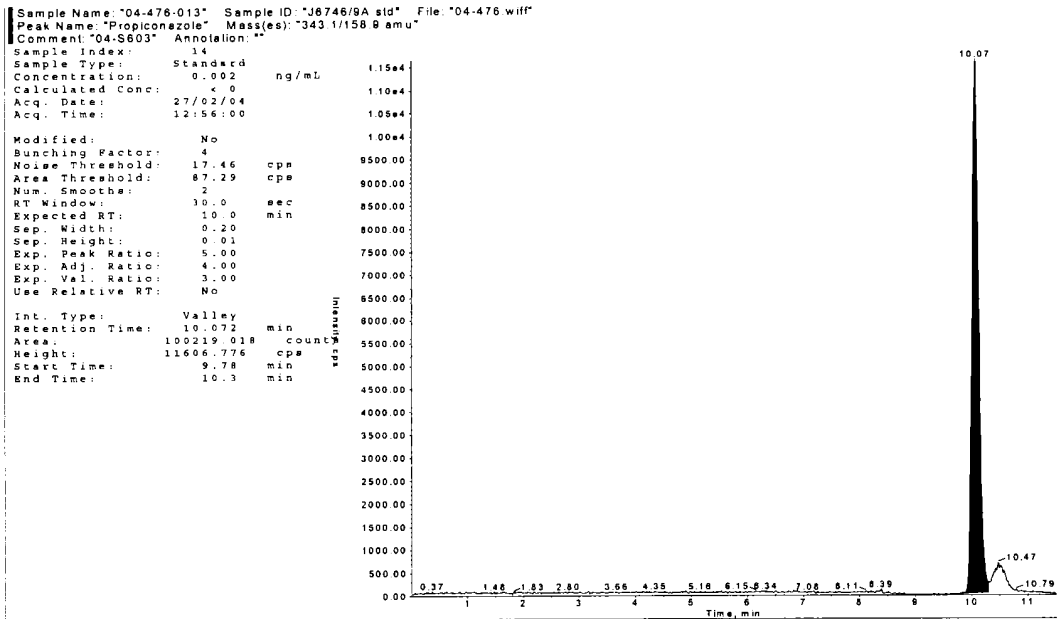
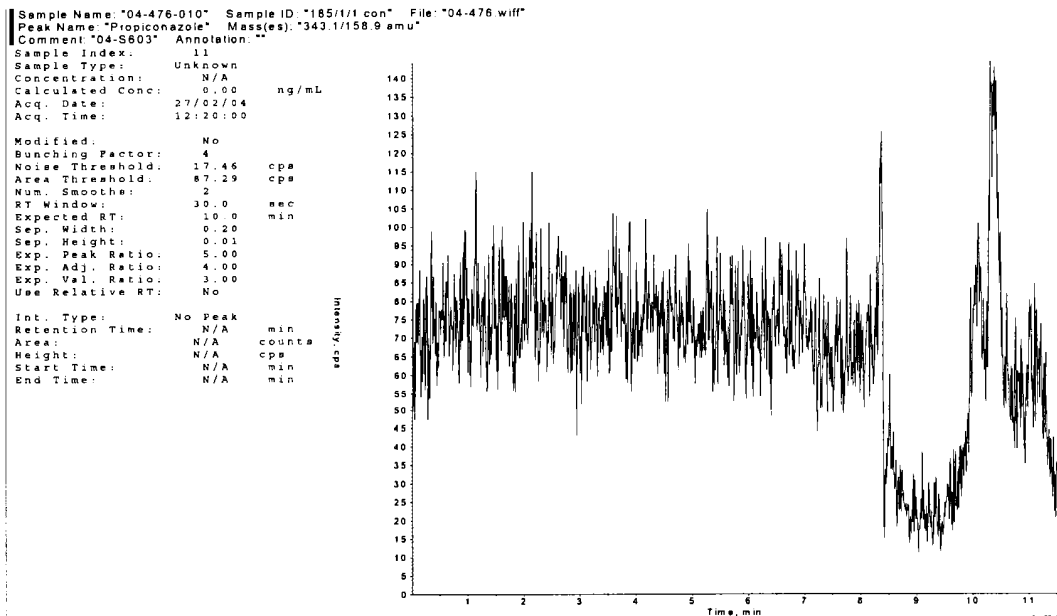
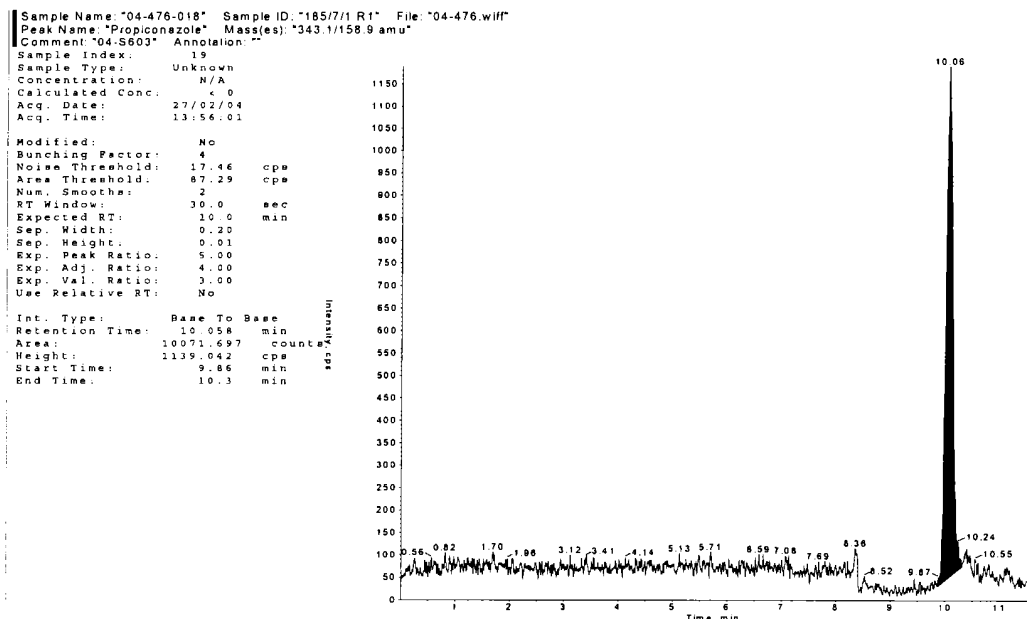


Figure 15 : Untreated oil seed rape at 0.02 g mL^{-1}



**Figure 16 : Untreated oil seed rape fortified at 0.01 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 100%.**



**Figure 17 : Untreated oil seed rape fortified at 0.1 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 96%.**

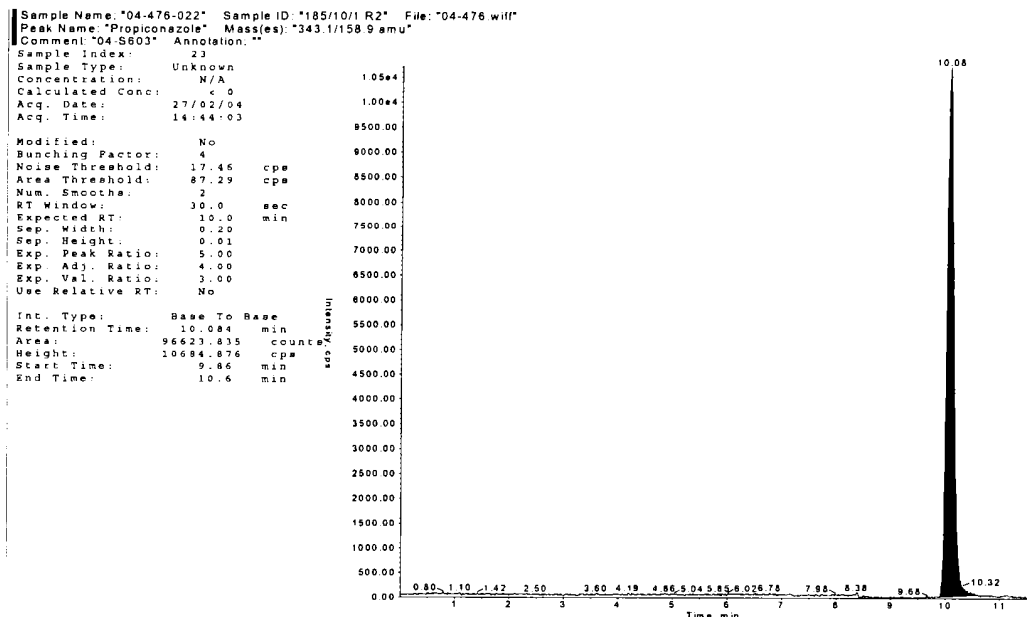


Figure 18 : 0.002 μ g mL⁻¹ Propiconazole standard.

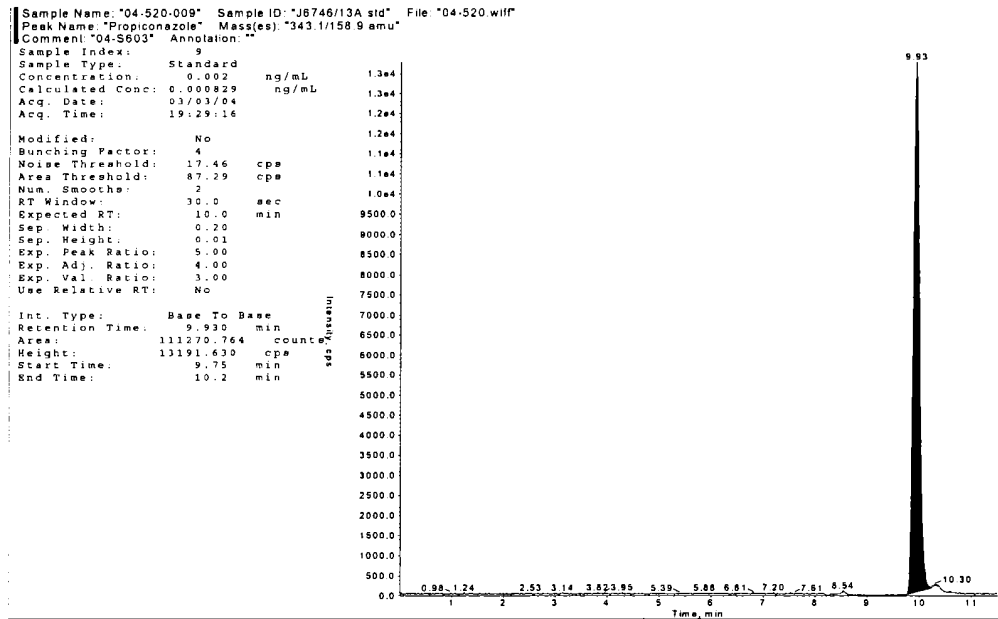
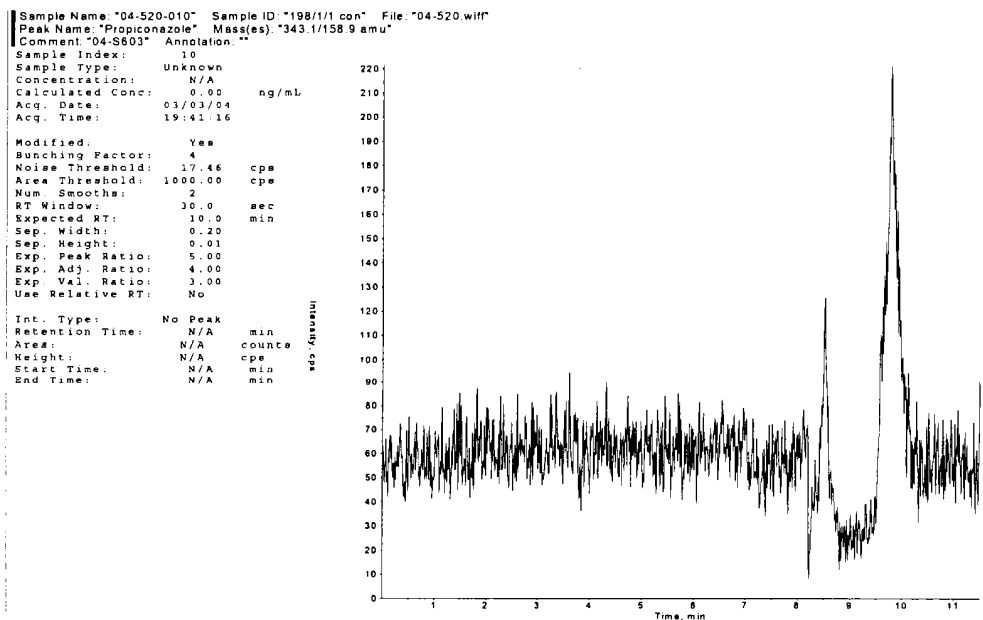
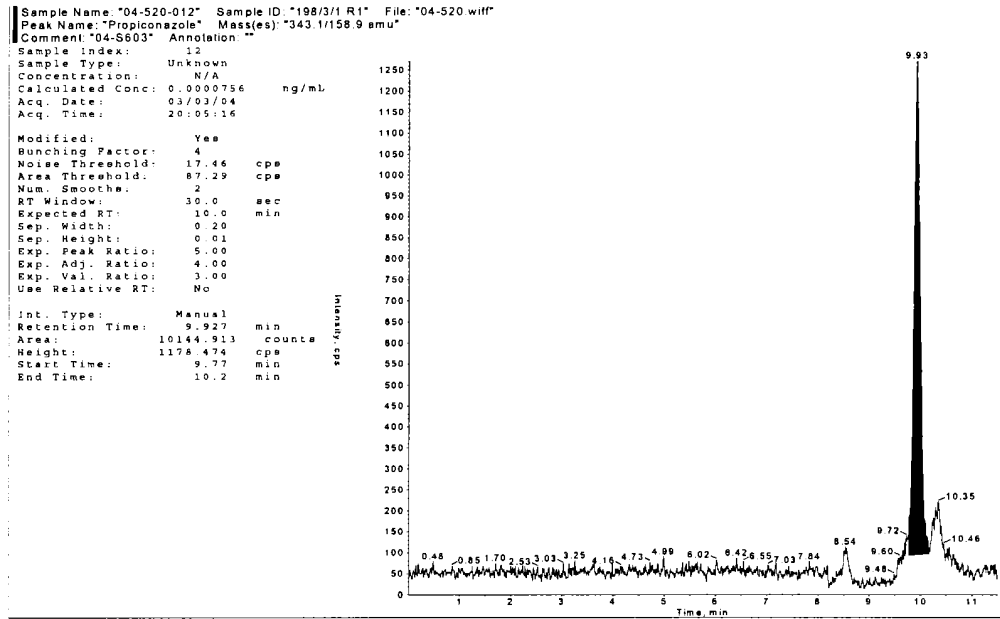


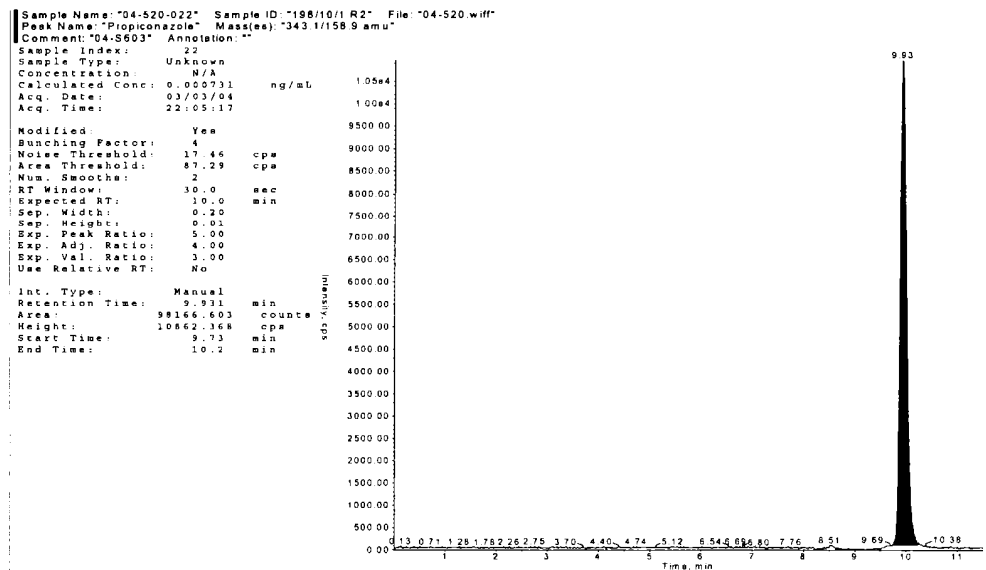
Figure 19 : Untreated winter barley forage at 0.02 g mL⁻¹.



**Figure 20 : Untreated winter barley forage fortified at 0.01 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 92%.**



**Figure 21 : Untreated winter barley forage fortified at 0.1 mg kg⁻¹.
Sample concentration = 0.02 g mL⁻¹. Recovery = 89%.**



Appendix 5: Detector Linearity Graphs

Figure 22 : LC-LC-MS/MS Detector Calibration Graph for Propiconazole, Intercept set to Zero

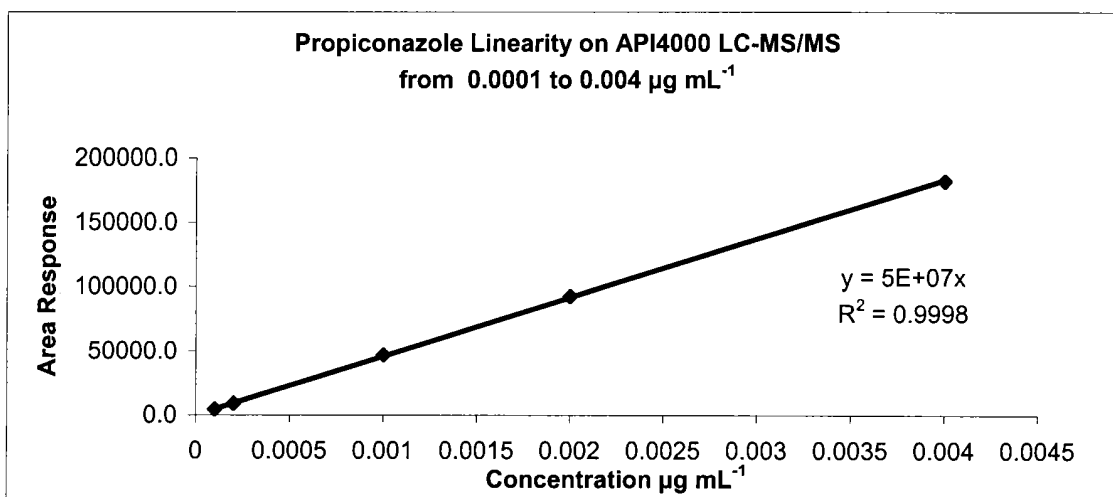
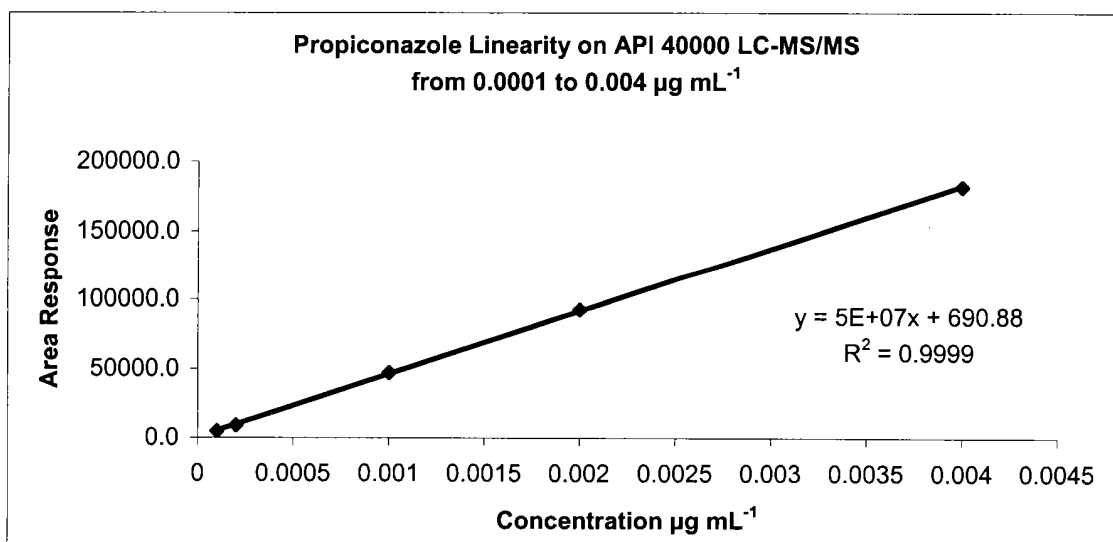


Figure 23 LC-LC-MS/MS Detector Calibration Graph for Propiconazole, No Intercept set



Appendix 6 : API 4000 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 Propiconazole

Infuse a standard solution of propiconazole (0.1 to $1.0 \mu\text{g mL}^{-1}$) in methanol: ultra pure water 80:20 v/v directly into the mass spectrometer interface at a rate at of $5 - 20 \mu\text{L min}^{-1}$. 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 343.

Using the Analyst software quantitative optimisation programme, tune the instrument for propiconazole, ensuring that the correct ions are selected (initial Q1 $m/z = 343$ and product ion $m/z = 160.1$). 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 propiconazole standards using a mobile phase of 55:45 (v/v) acetonitrile: ultra pure water + 0.2 % formic 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 24: Propiconazole Initial Product Scan (positive ionisation)

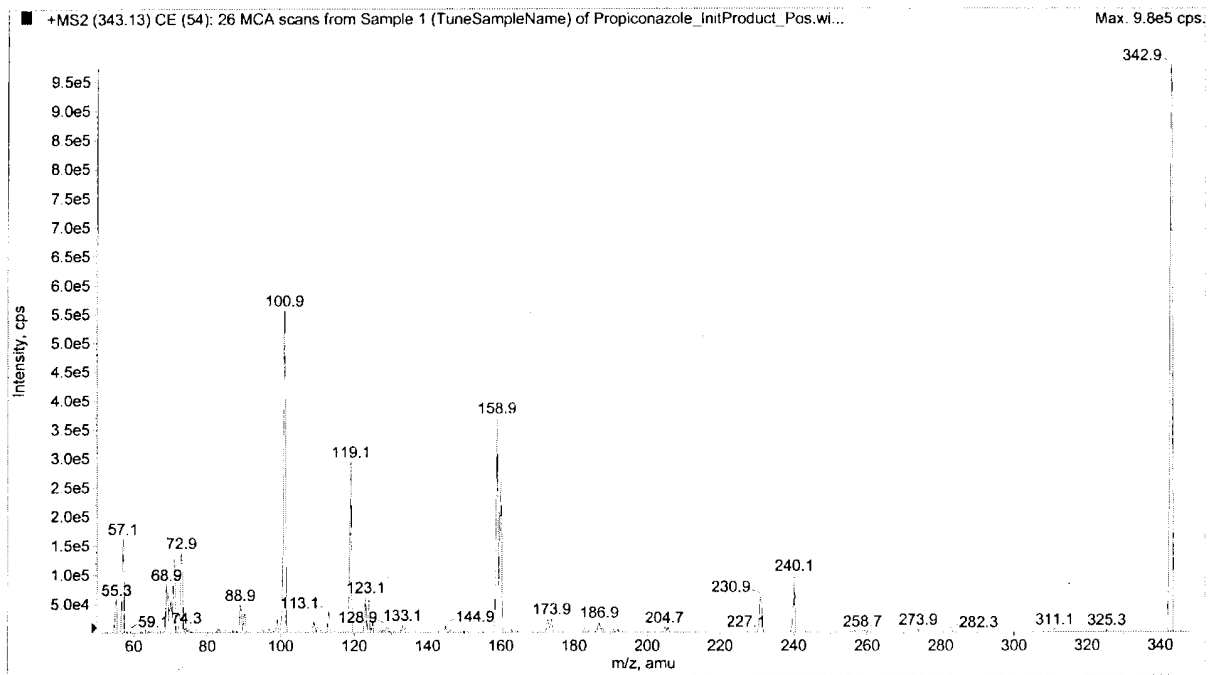
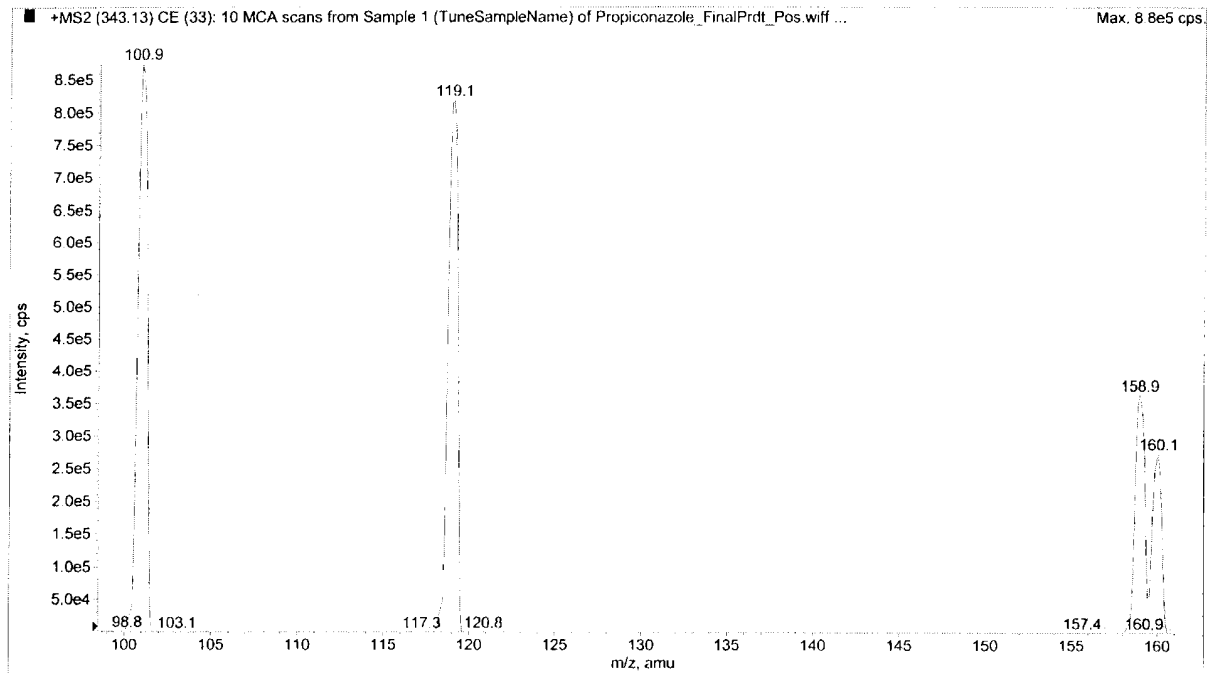
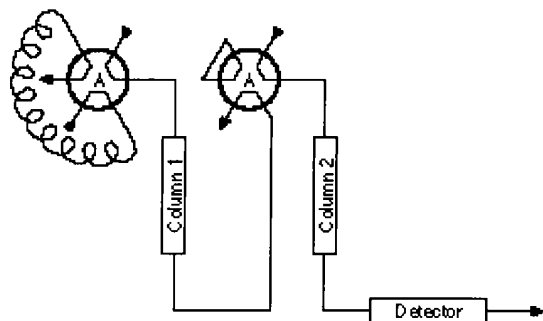


Figure 25 : Propiconazole Final Product Scan. Daughters of m/z = 342 (positive ionisation).

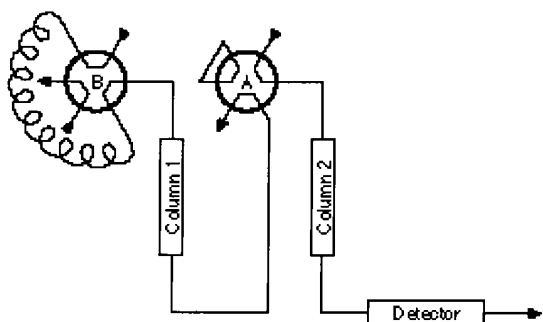


Appendix 7 : Configuration of Column Switching System

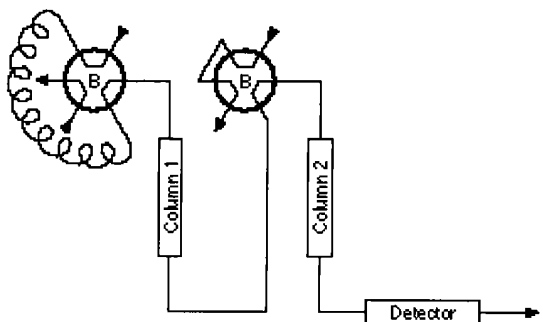
Figure 26 : Diagram of Column Switching Configuration



1) Sample loading



**2) Separation on first column
(T = 7.2-8.3 min)**



**3) Transfer of cut to second column
(T = 10.0-10.3 min propiconazole)**