R044686

R044686 - Analytical Method for the Determination of Total Chlorothalonil Residues in Crop Commodities by LC-MS/MS

Analytical Method

DATA REQUIREMENT(S): US EPA guideline OPPTS 860.1340

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Version	Summary of revisions
GRM005.03A	New method

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Abbreviations and symbols

Abbreviation	Definition
C	Celsius or Centigrade
CAS	Chemical Abstract Services
CFR	Code of Federal Regulations
EPA	Environmental Protection Agency (U.S.)
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (U.S.)
g	gram
GC	gas chromatography
GLP	Good Laboratory Practice
GRM	Global Residue Method
HPLC	high performance liquid chromatography
ID	identification
IUPAC	International Union of Pure and Applied Chemistry
Kg	kilogram
L	liter
LC	liquid chromatography
LC-MS/MS	liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
µg	microgram
µL	microliter
M	molar
mg	milligram
mL	milliliter
min	minute
MS	mass spectrometry
MSDS	material safety data sheets
MS/MS	tandem mass spectrometry/mass spectrometry
ms	millisecond
mV	millivolt
mw	molecular weight

Abbreviations and symbols (Continued)

Abbreviation	Definition
m/z	mass to charge ratio
N/A	not applicable
ND or nd	not detectable (below limit of detection)
ng	nanogram
No.	number
oz	ounce
ppb	parts per billion or micrograms per kilogram
ppm	parts per million or microgram per gram or milligrams per kilogram
pg	picogram
QAU	quality assurance unit
R^2 (or r^2)	square of correlation coefficient
RSD	relative standard deviation
RT	retention time
s	second
SD	standard deviation
SPE	solid phase extraction
USDA	United States Department of Agriculture
UV	ultraviolet
Vol	volume
Wt	weight
V	volt

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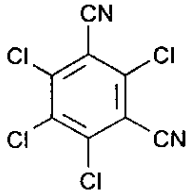
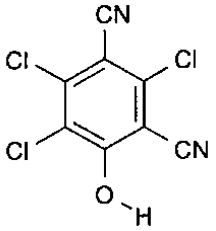
1.0 INTRODUCTION

1.1 Scope and chemical structures

Analytical Method GRM005.03A is suitable for the determination of chlorothalonil (R044686) and its metabolite, R182281, in soybean crop commodities and processed products. This method is based on the extraction procedures outlined in Syngenta Analytical Method GRM005.01A, "Chlorothalonil (R044686): Analytical Method for the Determination of Residues of Chlorothalonil and R182281 in Crops," but replaces the GC/MSD system with the LC-MS/MS technology for final determination of chlorothalonil and R182281 residues.

The limit of quantitation (LOQ) of the method has been established at 10 ppb chlorothalonil and 10 ppb R182281 for soybean seed, forage, hulls, oil and aspirated grain fractions, and at 50 ppb chlorothalonil and 10 ppb R182281 for soybean hay and meal. This method satisfies US EPA guideline OPPTS 860.1340. The chemical structures of chlorothalonil and R182281 are summarized as follows:

Compound Structure:	
Common Name:	Chlorothalonil
Code Name:	R044686
IUPAC Name:	2,4,5,6-tetrachloroisophthalonitrile
Chemical Name:	Tetrachloroisophthalonitrile
CAS Number:	1897-45-6
Molecular Formula:	$C_8Cl_4N_2$
Molecular Mass:	265.9

Compound Structure:	
Common Name:	Chlorothalonil Metabolite
Code Name:	R182281
IUPAC Name:	4-hydroxy-2, 5,6-trichloroisophthalonitrile
Chemical Name:	1,3-Benzenedicarbonitrile, 2,4,5 trichloro-6-hydroxy-
CAS Number:	28343-61-5
Molecular Formula:	$C_8HCl_3N_2O$
Molecular Weight:	247.5

1.2 Method summary

For solid crop commodities, approximately 10 grams of homogenized soybean crop sample are extracted with 100 mL of acetone:10 N sulfuric acid solution (95:5, v/v). After the sample is centrifuged or allowed to settle, a 2-mL aliquot is diluted to 10 mL with Optima water.

For soybean oil, approximately 1 mL of sample is extracted with methanol. After the sample is centrifuged, a 2-mL aliquot is diluted with 8 mL Optima water.

The samples are cleaned up using solid phase extraction (SPE). Chlorothalonil and R182281 are analyzed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS).

2.0 MATERIALS AND APPARATUS

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.1 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. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.2 Preparation of analytical standard solutions

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.

2.2.1 Primary stock solutions

Prepare two primary stock solutions: one containing chlorothalonil and one containing R182281. A 1.00 mg /mL primary stock solution is prepared by the following method:

Weigh 10.0 mg of analyte (chlorothalonil or R182281 corrected for purity) directly into a 10-mL volumetric flask. Add ethyl acetate to the mark on the flask.

2.2.2 Intermediate solutions

Prepare two intermediate solutions from the primary stock solutions: one containing chlorothalonil and one containing R182281.

Prepare an intermediate solution by aliquoting 2.5 mL of the primary stock solution (1.00 mg/mL) into a 50-mL volumetric flask. Add additional ethyl acetate to the mark on the flask. This intermediate solution will have a concentration of 50.0 µg/mL.

2.2.3 Fortification solutions

Sample fortification solutions should be prepared in ethyl acetate from the 50 µg/mL intermediate solution. Prepare one set of fortification solutions containing chlorothalonil, and prepare one set containing R182281.

Prepare a 1.00 µg/mL fortification solution by adding 1.00 mL of the intermediate solution to a 50-mL volumetric flask and then diluting to the mark with ethyl acetate. Prepare a 10.0 µg/mL fortification solution by adding 10.0 mL of the 50.0 µg/mL intermediate solution to a 50-mL volumetric flask and then diluting to the mark with ethyl acetate.

Store each solution frozen (-20 °C) in the dark. Standard solutions should be allowed to equilibrate to room temperature prior to use. It is recommended that standard solutions expire no later than 3 months after preparation.

2.2.4 Calibration standards for LC-MS/MS

At least 5 levels of calibration standards should be prepared to develop a calibration curve for calculation of sample residues. Standards for external calibration should be prepared in acetonitrile:water:formic acid (50:50:0.2, v/v/v). The following is a recommended preparation procedure and applies to both chlorothalonil and R182281 analytes:

Prepare a 50.0 ng/mL intermediate solution by aliquoting 0.100 mL of the 50.0 µg/mL fortification solution into a 100-mL volumetric flask. Dilute to the line with acetonitrile:water:formic acid (50:50:0.2, v/v/v).

Prepare calibration standards containing 0.250 ng/mL, 0.500 ng/mL, 1.00 ng/mL, 2.00 ng/mL, 5.00 ng/mL and 10.0 ng/mL by dilution of the 50.0 ng/mL intermediate solution with acetonitrile:water:formic acid (50:50:0.2, v/v/v) according to the following table:

Standard concentration (ng/mL)	Volume of 50.0 ng/mL intermediate solution used (mL)	Final Volume (mL)
0.100	0.2	100
0.250	0.5	100
0.500	0.5	50
1.00	1.0	50
2.00	2.0	50
5.00	5.0	50
10.0	10	50

Calibration standards should be stored in a freezer (-20 °C). An expiration date of 3 months is recommended. Typical LC-MS/MS chromatograms from analysis of chlorothalonil and R182281 standards are presented in Appendix 3 and 4.

2.3 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 MSDS.

Solvent	Ethyl Acetate	Formic Acid	Acetonitrile	Sulfuric Acid	Methanol	Acetone
Harmful Vapor	✓	✓	✓	✓	**X**	✓
Highly Flammable	✓	**X**	✓	**X**	**X**	✓
Harmful by Skin Absorption	✓	✓	✓	✓	**X**	✓
Irritant to Respiratory system & eye	✓	✓	✓	✓	**X**	✓
Syngenta Divisional Toxicity Class	SHC-B	SHC-C,S	SHC-C,S	SHC-C,S	SHC-B	SHC-B
OES Short Term (mg m^{-3})	N/A	N/A	105	N/A	250 ppm	750 ppm
OES Long Term (mg m^{-3})	400 ppm	9	70	0.2	200 ppm	500 ppm

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

3.0 ANALYTICAL PROCEDURE

The method procedures are summarized in flow chart form in Appendix 9.

3.1 Modifications and potential problems

For analysis of products with an uneven consistency such as soybean seed, a Polytron homogenizer should be used instead of an Omni mixer to ensure adequate extraction.

3.2 Sample preparation

After receipt, store samples frozen at -20 ºC. Samples should be prepared using an approved method of sample preparation for residue analysis. See details in the most current revision of Syngenta SOP 7.21 "Preparation of Crop Samples for Residue Analysis."

3.3 Sample Fortification

At least one untreated control and two control samples fortified with known amounts of chlorothalonil and/or R182281 should be analyzed with each sample set to verify method performance and allow recovery corrections to be made if desired.

To calculate the volume of fortification solution to be added, or the theoretical fortification concentration, use the following equation:

$$Fortification\ conc.(\ ppb\ ) = \left(\frac{fort.sol.conc.(\ \mu g\ /\ mL\ )\ x\ Vol.of\ \ sol.added\ (\ mL\ )}{sample\ weight\ \ (\ g\ )} \right) \left(\frac{1000\ ng}{1\ \mu g} \right)$$

Let each sample stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. **Note: No control/recovery samples will be analyzed when the method is used for tolerance enforcement purpose.**

3.4 Extraction for analysis of soybean seed and soybean hulls

a) Weigh out a 10-gram representative subsample into a 250-mL glass bottle. Fortify samples as required at this point.

b) Carefully measure 100 mL of acetone:5 M (10N) sulfuric acid solution (95:5, v/v) into the glass bottle and homogenize the mixture for 1 minute using a Polytron homogenizer at medium speed.

c) Allow sample to settle for approximately 15 minutes.

d) Once the sample has settled, transfer 2 mL of the liquid extract into a 15-mL screw-cap test tube. Add 8 mL of Optima water. Mix the samples by inversion. Continue to Section 3.8 Solid Phase Extraction. Store extracts refrigerated if Section 3.8 cannot be performed immediately.

3.5 Extraction for analysis of soybean forage, hay and meal

a) Weigh out a 10-gram representative subsample into a 250-mL glass bottle. Fortify samples as required at this point.

b) Carefully measure 100 mL of acetone:5 M (10N) sulfuric acid solution (95:5, v/v) into the 250-mL glass bottle and homogenize the mixture for 3 minutes using an Omni mixer.

c) Centrifuge the sample for 4 minutes at 3000 RPM.

d) Transfer 2 mL of the supernatant into a 15-mL screw-cap test tube. Add 8 mL of Optima water. Mix the samples by inversion. Continue to Section 3.8 Solid Phase Extraction. Store extracts refrigerated if Section 3.8 cannot be performed immediately.

3.6 Extraction for analysis of soybean oil

a) Measure 1 mL of soybean oil into a 15-mL glass bottle. Fortify samples as required at this point.

b) Carefully add 3 mL of methanol into the 15-mL glass bottle and shake vigorously for 1 minute.

c) Centrifuge the bottle for 4 minutes at 3000 RPM.

d) Using a Pasteur pipette, carefully transfer all 3 mL of the upper methanol layer to a separate 15-mL graduated, glass bottle. Cap.

e) Repeat steps b, c and d two more times resulting in an extract volume of approximately 9 mL of methanol. Record actual volume.

f) Transfer 2 mL of the methanol extract into a 15-mL screw-cap test tube. Add 8 mL of Optima water. Mix the samples by inversion. Continue to Section 3.8 Solid Phase Extraction. Store extracts refrigerated if Section 3.8 cannot be performed immediately.

3.7 Extraction for analysis of soybean aspirated grain fractions

a) Weigh out a 2-gram representative subsample into a 50-mL glass bottle. Fortify samples as required at this point.

b) Carefully add 20 mL of acetone:5 M sulfuric acid solution (95:5, v/v) into the 50-mL glass bottle and homogenize the mixture for 3 minutes using an Omni mixer.

c) Centrifuge the sample for 4 minutes at 3000 RPM.

d) Transfer 2 mL of the supernatant into a 15-mL screw-cap test tube. Add 8 mL of Optima water. Mix the samples by inversion. Continue to Section 3.8 Solid Phase Extraction. Store extracts refrigerated if Section 3.8 cannot be performed immediately.

3.8 Solid phase extraction procedure

a) Take one Varian Bond Elut C_{18} solid phase extraction column for each sample to be analyzed and place on a suitable vacuum manifold. Add 3 mL of methanol to each column. Elute the methanol at a rate of approximately 2 mL/minute. Do not allow the cartridges to become dry. Add 4 mL of Optima water to each column. Elute the water at a rate of approximately 2 mL/minute. Do not allow the cartridges to become dry. Discard column eluate.

b) Load 10 mL of extract onto the appropriate column. Maintain the flow rate at approximately 2 mL/min and discard the eluate. Close the stopcock each time the solvent level approaches the cartridge bed frit.

Note: It is recommended that the graduations on each batch of test tubes are checked prior to use. Pipette 5 mL of solvent into the tube to confirm that the solvent meniscus corresponds to the 5 mL graduation mark on the tube.

c) Place suitable 15-mL graduated test tubes under each port, as required, in the manifold rack. Add 5 mL of acetonitrile:water:formic acid (40:60:0.2, v/v/v) to each column to elute R182281. Collect the eluate at a rate of approximately 2 mL/min. Record each final volume. See Section 4.0 for instrument installation and operating conditions for analysis of R182281.

d) Place a new set of suitable 15-mL graduated test tubes under each port, as required, in the manifold rack. Add 5 mL of acetonitrile:water:formic acid (70:30:0.2, v/v/v) to each column to elute chlorothalonil. Collect the eluate at a rate of approximately 2 mL/min. Record the final volume. See Section 4.0 for instrument installation and operating conditions for analysis of chlorothalonil.

3.9 Time required for analysis

The methodology is normally performed with a batch of 9-12 samples. One skilled person can complete the analysis of 2 batches of samples (18-24) in 8 working hours.

3.10 Method stopping points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will

validate any work flow interruptions. Samples may be stored in a refrigerator or at room temperature in sealed containers if the analysis cannot be completed in a single day.

4.0 FINAL DETERMINATION

The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimization 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.

A separate analysis is performed for each compound as a result of different SPE procedures.

4.1 Instrument description

LC-MS/MS System	:	Sciex API4000 LC-MS/MS with Shimadzu LC-10AD VP HPLC Pumps, Shimadzu SCL-10A VP Controller and Perkin Elmer Series 200 Autosampler
Detector	:	Applied Biosystems API 4000 Triple Quadrupole with Analyst Software (version 1.4)

4.2 Chromatography conditions

HPLC Pump:	Shimadzu LC-10AD VP HPLC Pumps
Mobile Phase A:	0.2% formic acid in acetonitrile
Mobile Phase B:	0.2% formic acid in water
Flow Rate:	0.500 mL/min
Column:	Phenomenex Luna 3µ C18 100Å (30 x 2.00 mm 3 micron)

4.2.1 Chlorothalonil in soybean seed, hulls, meal, oil and aspirated grain fractions

Injection Vol. 10 µL
Retention Time: ~2.25 minutes
Run Time: 6.5 minutes
Divert Valve 2.0 minutes, LC/MS/MS
2.6 minutes, Waste
(for aspirated grain fractions, 1.5 min LC/MS/MS and 2.5 min waste)

Mobile Phase Composition

Time (min)	A% (0.2% formic acid in acetonitrile)	B% (0.2% formic acid in water)	Gradient curve
0.0	60	40	Initial
3.0	90	10	Linear
5.0	90	10	Isocratic
5.1	60	40	Linear
6.5	60	40	Isocratic

4.2.2 Chlorothalonil in soybean forage and soybean hay

Injection Vol. 10 µL
Retention Time: ~3.21 minutes
Run Time: 7.5 minutes
Divert Valve 2.9 minutes, LC/MS/MS
3.5 minutes, Waste

Mobile Phase Composition

Time (min)	A% (0.2% formic acid in acetonitrile)	B% (0.2% formic acid in water)	Gradient curve
0.0	40	60	Initial
4.0	60	40	Linear
5.0	100	0	Linear
6.0	100	0	Isocratic
6.1	40	60	Linear
7.5	40	60	Isocratic

4.2.3 R182281 in all soybean matrices

Injection Vol. 10 µL
Retention Time: ~3.8 minutes
Run Time: 6.5 minutes
Divert Valve 3.0 minutes, LC/MS/MS
4.5 minutes, Waste

Mobile Phase Composition

Time (min)	A% (0.2% formic acid in acetonitrile)	B% (0.2% formic acid in water)	Gradient curve
0.0	40	60	Initial
4.0	80	20	Linear
5.0	100	0	Linear
5.1	40	60	Linear
6.5	40	60	Isocratic

4.3 Chlorothalonil mass spectrometer conditions

Detection: Heated Nebulizer APCI Negative Polarity Multiple Reaction Monitoring (MRM) as follows:

Q1 MS	Q3 MS	Dwell Time
m/z = 245	m/z = 245	800 ms

MS/MS Conditions:

Source Temperature:	550
CAD Gas:	8
Curtain Gas	25
GS1	30
GS2	0
NC	-3
Q1 Resolution	High
Q3 Resolution	High
Ion Energy 1	-0.500
Ion Energy 2	-0.700
CEM	2200.0
Deflector	50.0
Settling Time	0.0000 msec
Declostering Potential	-70
Enhance Potential	-10
Collision Energy	-30
CXP	-4

4.4 R182281 mass spectrometer conditions

Detection: TurboSpray Ionization Negative Polarity Multiple Reaction Monitoring (MRM) as follows:

Q1 MS	Q3 MS	Dwell Time
m/z = 245	m/z = 182	900 ms

MS/MS Conditions:

Source Temperature	450
CAD Gas	6
Curtain Gas	20
GS1	40
GS2	40
IS	-1500.00
Q1 Resolution	Unit
Q3 Resolution	Unit
Ion Energy 1	-1.000
Ion Energy 2	-0.300
CEM	2000.0
Deflector	400.0
Setting Time	0.0000 msec
Declostering Potential	-68
Enhance Potential	-10
Collision Energy	-39
CXP	-14

Note: The MS settings above should be used as guidelines only. For optimal results, a tune should be performed by the analyst.

Data Acquisition: Raw area counts are downloaded from the Analyst data collection system (Version 1.4) to Microsoft Excel to calculate the final results.

5.0 CALCULATION OF RESULTS

This method was developed using a multipoint calibrating procedure with no matrix matched calibration. Residue weight concentrations may be calculated in parts per billion (ppb) or ng/g for each sample as follows.

a) Prepare chlorothalonil/R182281 standard solutions over a concentration range appropriate to the expected residues in the samples.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to the analyte of interest. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + b$$

Where y is the chromatography peak area, x is the analyte concentration in ng/mL in the final fraction, m is the slope of the line of best fit ("X-variable 1" in MS Excel) and b is the intercept value. An example of this equation generated using the experimental values of m and b should be included in the raw data, as should the "r^2" value for the regression. A typical calibration curve for chlorothalonil is shown in Appendix 5. A typical calibration curve for R182281 is shown in Appendix 6.

e) Re-arrangement for *x* gives

$$Chlorothalonil\ /\ R182281\ concentration\ (ng/mL)\ in\ final\ fraction = x = \frac{y - b}{m}$$

f) To determine the residue concentration (ng/g or ppb) of chlorothalonil/R182281 in the samples, use the following equation:

$$Sample\ conc.(ppb)^{*} =$$

$$\frac{conc.\ from\ curve\ in\ ng/mL\ x\ Aliquot\ Factor\ x\ Dilution\ Factor\ x\ Final\ Vol.\ (mL)}{sample\ weight\ (g)}$$

* For soybean oil, the chlorothalonil/R182281 residue will be in ng/mL.

6.0 UNTREATED CONTROL AND RECOVERY EXPERIMENTS

If untreated control samples are available, untreated control samples should be analyzed with each set of samples to verify that samples are free of analyte contamination. A minimum of one control should be analyzed with each batch of samples.

At least two recovery samples (untreated samples accurately fortified with a known amount of analyte prior to extraction) should also be analyzed alongside each batch of samples. The recovery levels should be appropriate to the residue levels expected. To determine the recovery percentage, determine the theoretical fortification amount using the equation in Section 3.0, and calculate the final residue values found in the fortified samples using the equations in Section 5.0 above.

Determine the recovery factor by first subtracting the residue found in the control sample, if any, from the residue found in the recovery sample. Calculate the recovery percentage (R%) by the following equation:

$$R\% = \frac{residue\ found\ in\ sample\ (ppb) - residue\ found\ in\ control\ (ppb)}{theoretical\ fortification\ amount\ (ppb)}\ x\ 100$$

Recovery efficiency is acceptable when the mean values are between 70% and 120%.

7.0 SPECIFICITY

7.1 Confirmatory transition

No confirmatory analysis was developed.

7.2 Matrix

No ion suppression or enhancement is found for analysis of chlorothalonil and R182281 in the selected crop commodities.

7.3 Reagent and solvent interference

Using high purity solvents and reagents, no interference should be encountered.

7.4 Labware interference

This method uses disposable labware where possible. All reusable glassware should be detergent washed and then rinsed with acetone and in-house deionized water prior to use.

8.0 METHOD TRIALS

A method trial (Study T002291-03, Reference 2) was carried out on the procedures described in this method for analysis of chlorothalonil and R182281 in seven matrices: soybean seed, forage, hay, hulls, meal, oil and aspirated grain fractions. The following discussion is based on the method trial data from Study T002291-03.

8.1 Accuracy and precision

For each of the seven matrices, three untreated control samples were fortified at the LOQ (10 or 50 ppb) and three at 10x the LOQ (100 or 500 ppb). Soybean seed, forage, hay, hulls and aspirated grain fractions were also fortified at various higher levels up to 3000x the LOQ. The procedural recoveries for chlorothalonil ranged from 70.3% to 114% for a total of 57 samples with an average of 97.8 ± 9.90% (RSD = 10.1%). The procedural recoveries for

R182281 ranged from 70.8% to 126% for a total of 53 samples with an average of 97.6 ± 14.2% (RSD = 14.5%). These results demonstrate residues of chlorothalonil can be determined in crop commodities with good accuracy and reproducibility. Typical LC-MS/MS chromatograms from analysis of crop commodities are presented in Appendix 5.

8.2 Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) is defined as the lowest analyte concentration in a sample at which the methodology has been validated. Generally, for accurate quantitation, 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 quantitation (LOQ) of the method has been established at 10 ppb chlorothonil and 10 ppb R182281 for soybean seed, forage, hulls, oil and aspirated grain fractions, and at 50 ppb chlorothalonil and 10 ppb R182281 for soybean hay and meal.

8.3 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 (LOD) is 2.5 pg for chlorothalonil and R182281. An injection of 2.5 pg of chlorothalonil standard (0.250 ng/mL with 10 µL injection volume), can be reliably quantified with a signal-to-noise ratio of significantly greater than 4:1.

8.4 Detector linearity

For accurate quantitation of residue concentrations, analyses should be carried out within the linear range of the detector response. Linearity of the detector is assured by the development of a calibration curve with each batch injected. It has been shown that the LC-MS/MS detector responses are generally linear in the range from 2.5 to 100 pg injected on column for chlorothalonil. These are equivalent to 0.250 to 10.0 ng/mL of chlorothalonil or R182281.

9.0 LIMITATIONS

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

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of residues of chlorothalonil and R182281 in soybean processed product and crop commodities. This method satisfies US EPA guideline OPPTS 860.1340.

11.0 REFERENCES

1. Chaggar, S. (2006), Syngenta Analytical Method GRM005.01A, "Chlorothalonil (R044686): Analytical Method for the Determination of Residues of Chlorothalonil and R182281 in Crops"

2. Boatwright, M. (2009) Analytical Phase Report, Syngenta Task No. T002291-03, "Chlorothalonil-Magnitude of the Residues in or on Soybean"

APPENDICES SECTION

APPENDIX 1 Apparatus

- Balance, Analytical, capable of accurately weighing 10.00 grams
- Centrifuge capable of 4000 RPM
- Omni International mixer homogenizer
- Polytron homogenizer
- Weigh boats/weighing dish
- Volumetric flasks, 10, 50 mL, 100 mL
- Jars, amber glass with Teflon lined cap: 25 mL, 50 mL, 100 mL and 125 mL
- Polypropylene centrifuge tubes, 250 mL
- Polypropylene centrifuge bottles, 200 mL
- 15-ml culture tubes, screw-cap with Teflon-lined lids
- 15-ml culture tubes, graduated
- SPE cartridge, C_{18} Varian HF Bond Elut, 500 mg, or equivalent
- Multiple port solid phase extraction vacuum manifold equipped with nylon stopcocks
- Vial for HPLC injection, 2-mL glass
- Disposable Volumetric pipettes, 1, 2, 5, 10 mL
- Disposable Pasteur pipettes with 2-mL bulb
- Graduated cylinders, 10, 100, 1000 mL

APPENDIX 2 Reagents

US suppliers

Acetone	Fisher No. A929-4, VWR No. BJAH010-4
Acetonitrile	Fisher No. A996-4, VWR No. BJAH015-4
Methanol	Fisher No. A454-4, VWR No. BJAH230-4
Water	Fisher No. W7-4, VWR No. BJAH365-4
Formic Acid	Fisher No. A118P-500, VWR No. JT0128-1
Sulfuric Acid	Fisher No. A300-212, VWR No. JT9681-5

Preparation of reagents

Acetonitrile:Water:Formic Acid (70:30:0.02, v/v/v)

Prepared by mixing 699 mL of acetonitrile with 299 mL of water, and then adding 2 mL of formic acid

Acetonitrile:Water:Formic Acid (50:50:0.02, v/v/v)

Prepared by mixing 499 mL of acetonitrile with 499 mL of water, and then adding 2 mL of formic acid

Acetonitrile:Water:Formic Acid (40:60:0.02, v/v/v)

Prepared by mixing 399 mL of acetonitrile with 599 mL of water, and then adding 2 mL of formic acid

10 N Sulfuric Acid Solution

Prepared by adding 272 mL concentrated sulfuric acid solution to 728 mL of water

Acetone:5 M (10 N) Sulfuric Acid Solution (95:5, v/v)

Prepared by adding 100 mL of 10 N sulfuric acid solution to 1900 mL of acetone

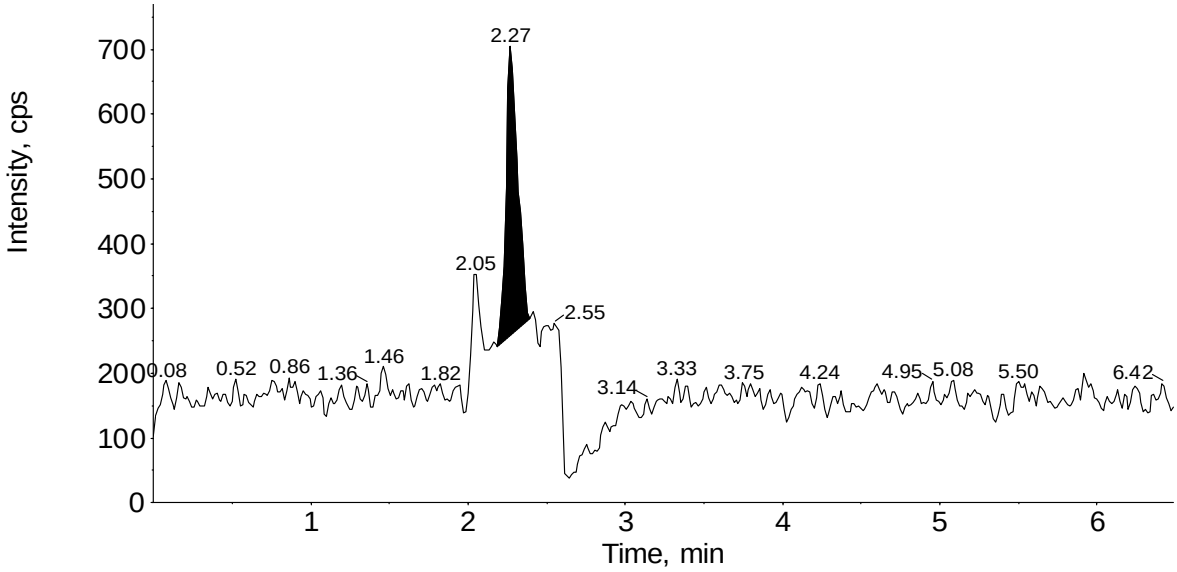
UK suppliers

Unknown

APPENDIX 3 Representative Chromatograms

Figure 1 Typical LC-MS/MS Chromatograms for Chlorothalonil Standards

Standard (0.250 ng/mL), 10 µL injection volume, 2.5 pg of chlorothalonil injected
peak area = 2296.7



Standard (0.500 ng/mL), 10 µL injection volume, 5.0 pg of chlorothalonil injected
peak area = 4784.2

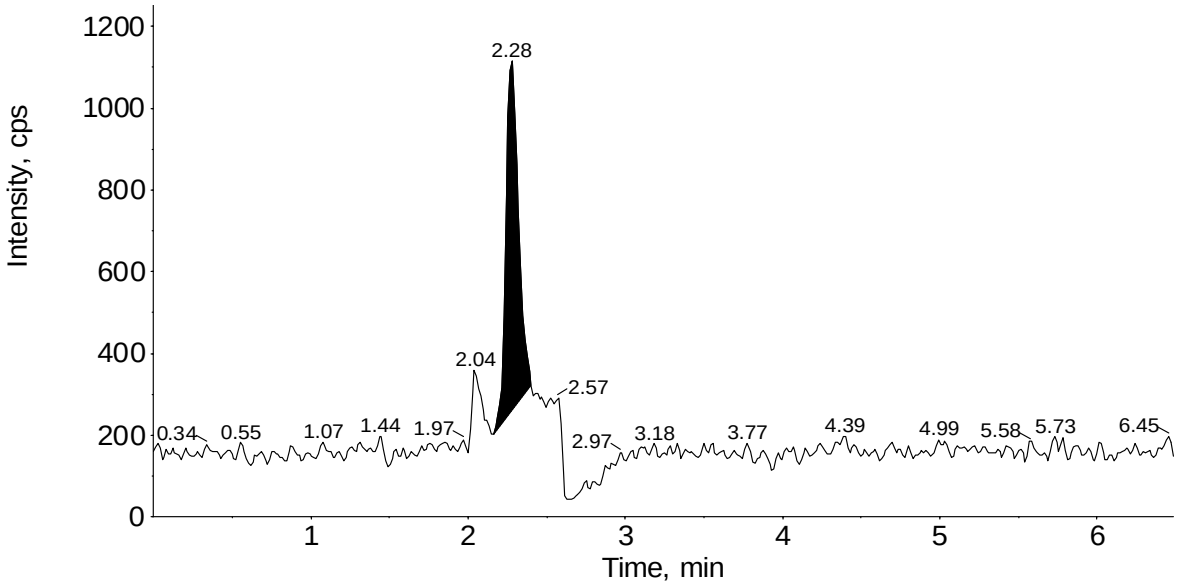
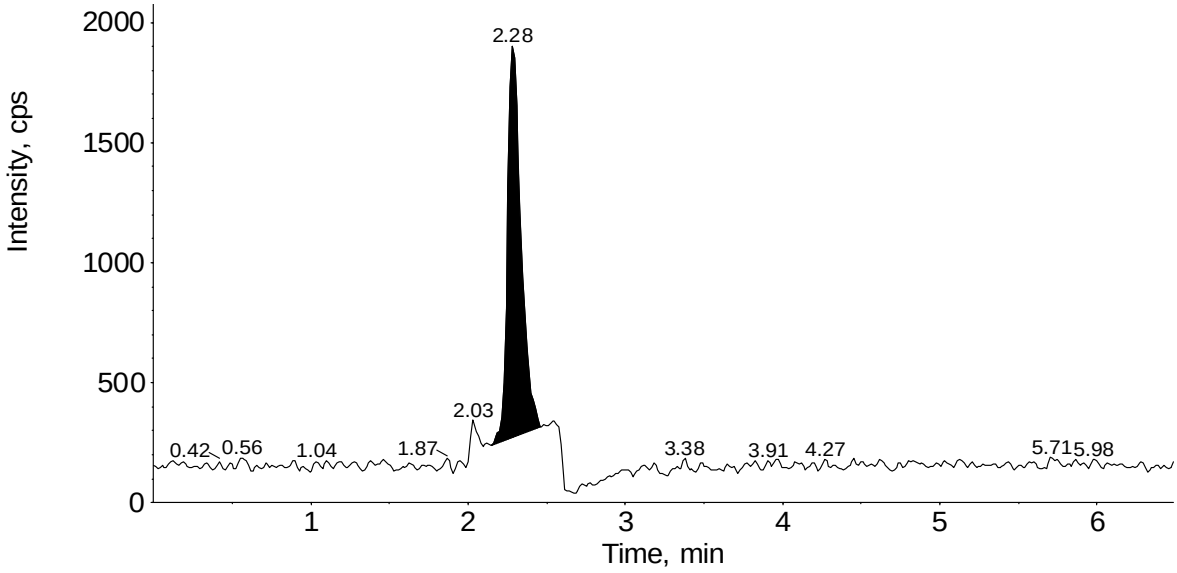


Figure 1 Typical LC-MS/MS Chromatograms for Chlorothalonil Standard (Continued)

Standard (1.00 ng/mL), 10 µL injection volume, 10 pg of chlorothalonil injected
peak area = 9769.4



Standard (2.00 ng/mL), 10 µL injection volume, 20 pg of chlorothalonil injected
peak area = 19322.91

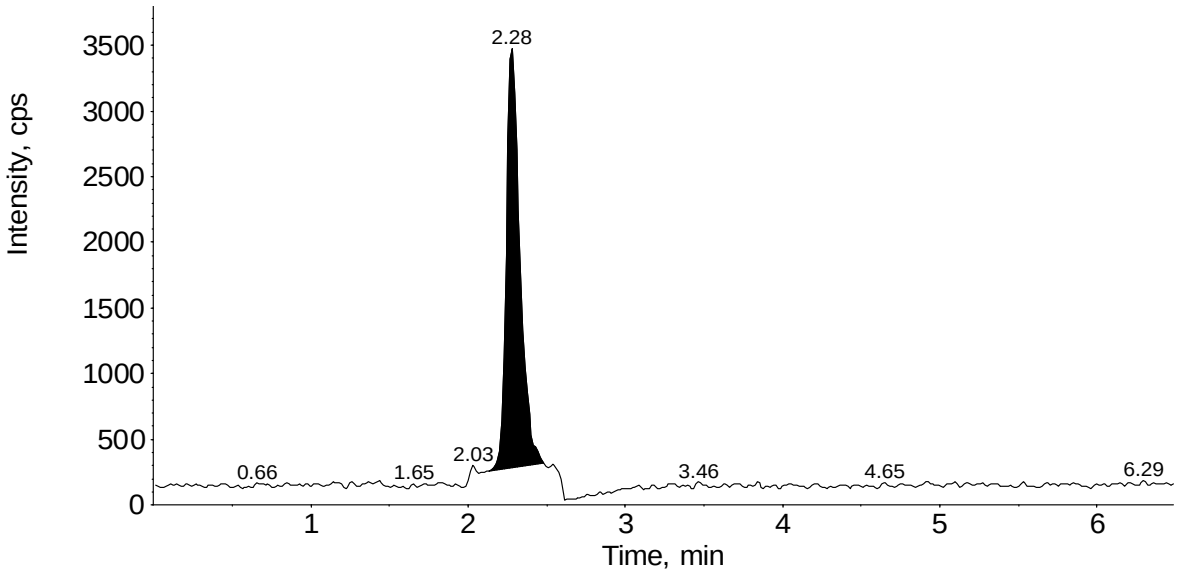
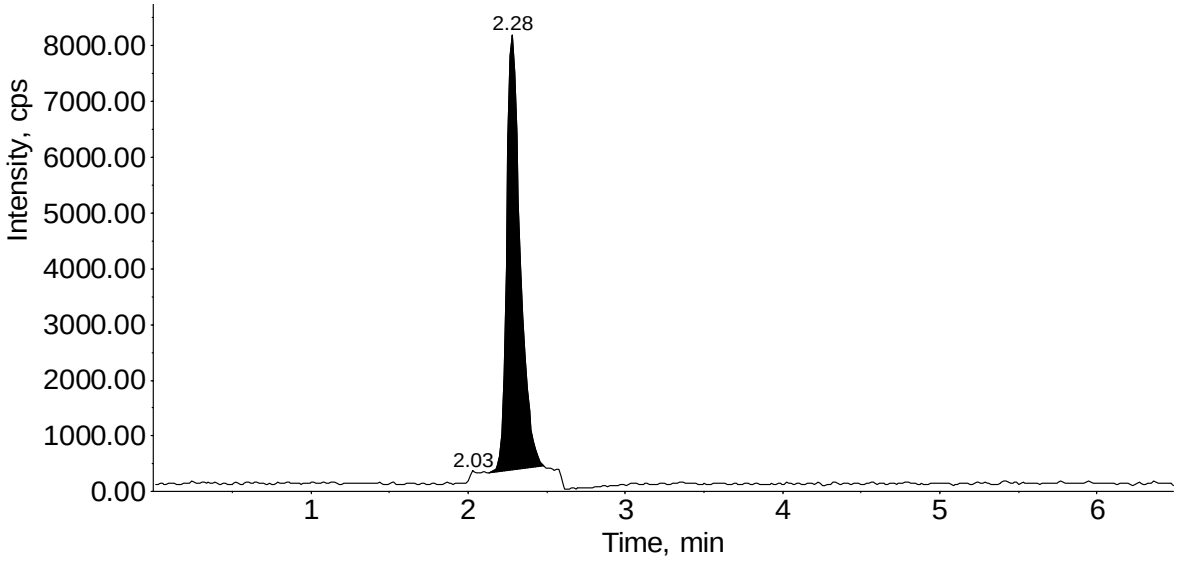


Figure 1 Typical LC-MS/MS Chromatograms for Chlorothalonil Standard (Continued)

Standard (5.00 ng/mL), 10 µL injection volume, 50 pg of chlorothalonil injected
peak area = 46800.93



Standard (10.0 ng/mL), 10 µL injection volume, 100 pg of chlorothalonil injected
peak area = 96319.27

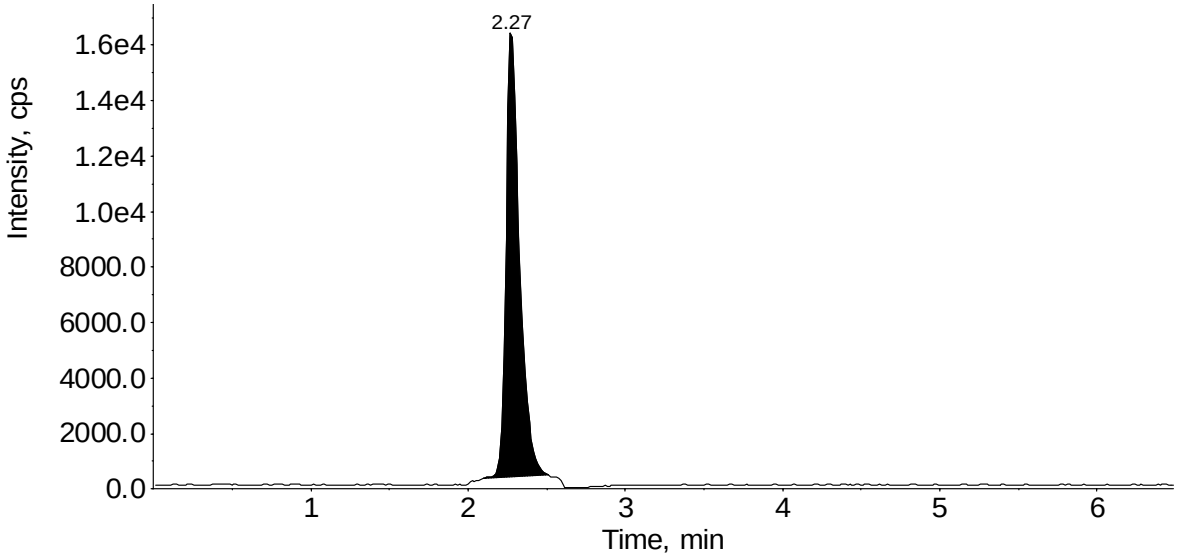
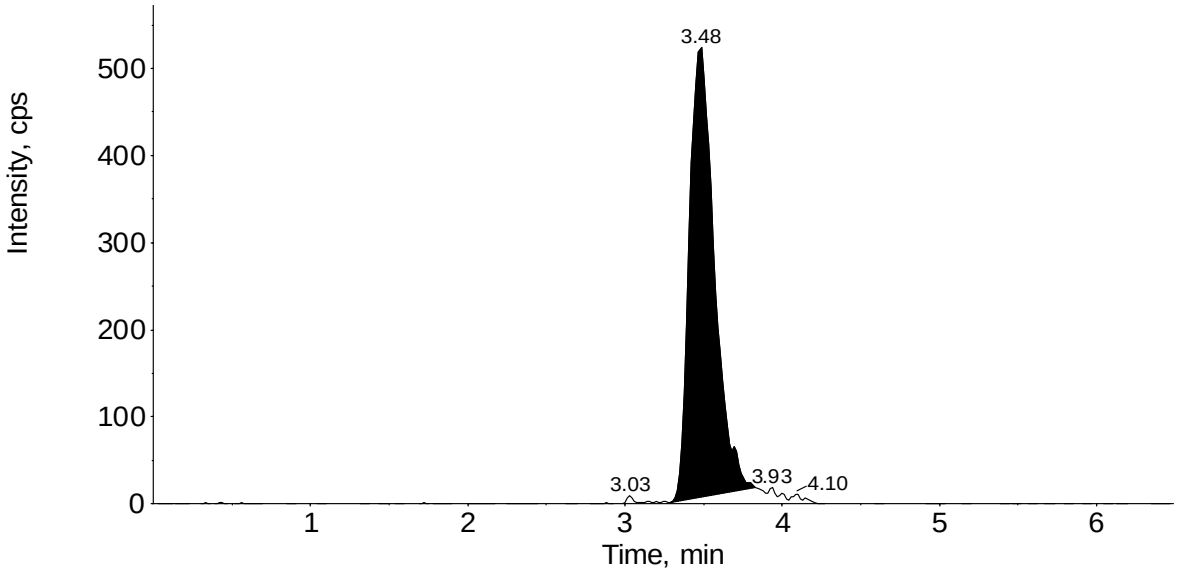


Figure 2 Typical LC-MS/MS Chromatograms for R182281 Standards

Standard (0.250 ng/mL), 10 µL injection volume, 2.5 pg of R182281 injected
peak area = 5614.1



Standard (0.500 ng/mL), 10 µL injection volume, 5.0 pg of R182281 injected
peak area = 11180.8

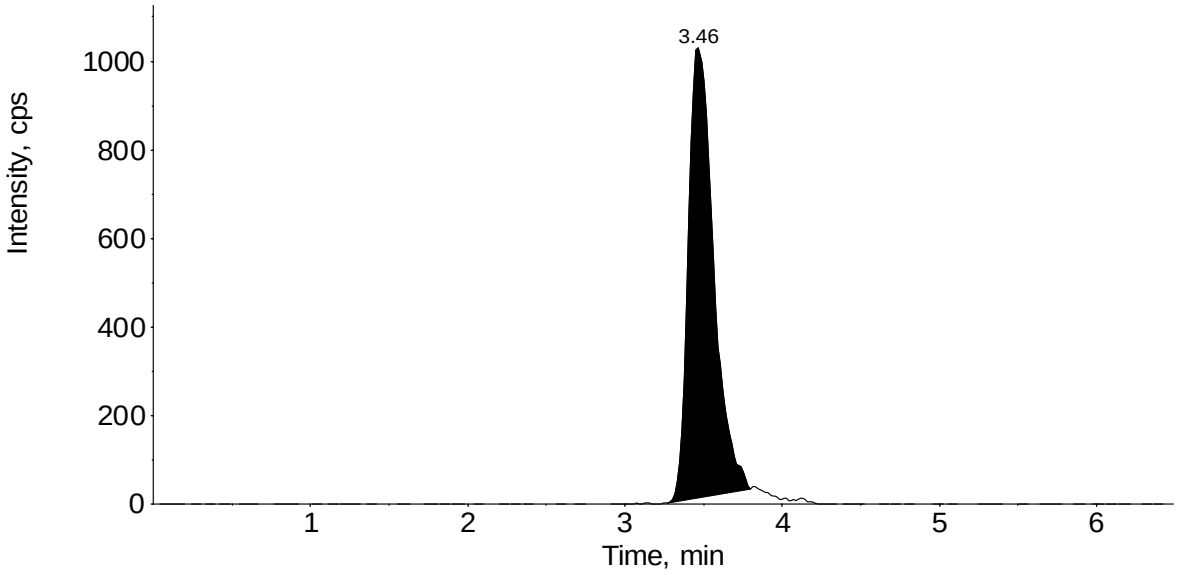
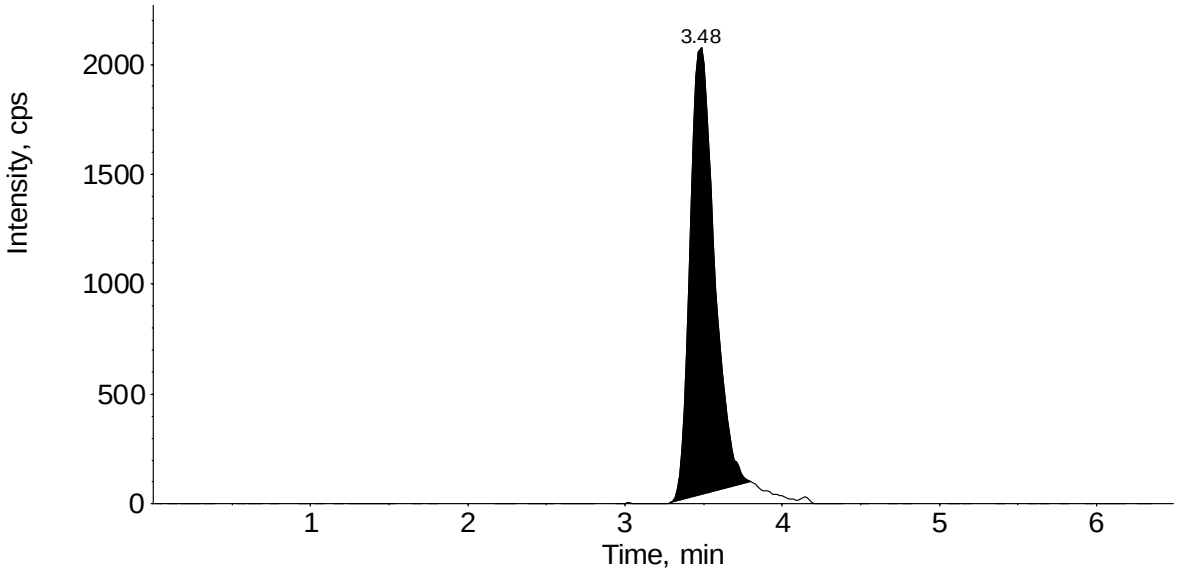


Figure 2 Typical LC-MS/MS Chromatograms for R182281 Standards (Continued)

Standard (1.00 ng/mL), 10 µL injection volume, 10 pg of R182281 injected
peak area = 21432.4



Standard (2.00 ng/mL), 10 µL injection volume, 20 pg of R182281 injected
peak area = 41778.7

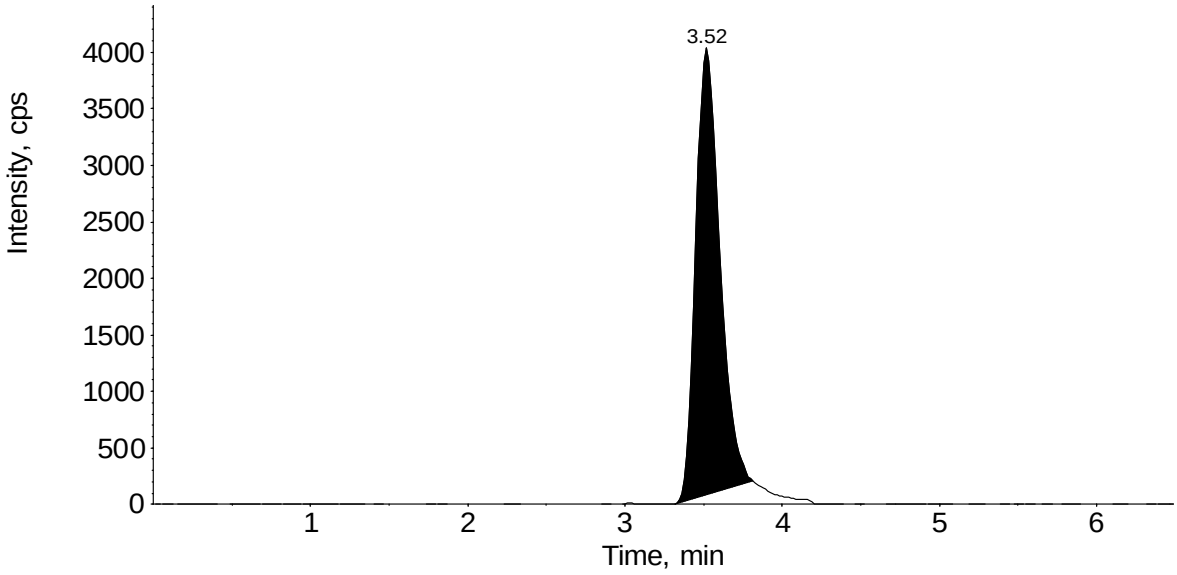
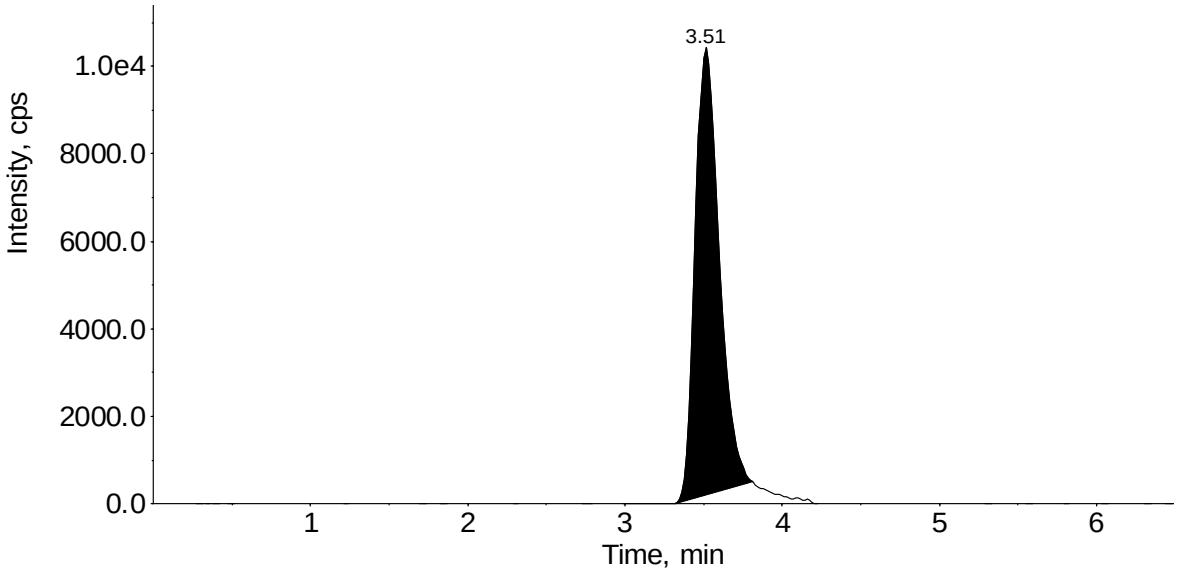


Figure 2 Typical LC-MS/MS Chromatograms for R182281 Standards (Continued)

Standard (5.00 ng/mL), 10 µL injection volume, 50 pg of R182281 injected
peak area = 107804.4



Standard (10.0 ng/mL), 10 µL injection volume, 100 pg of R182281 injected
peak area = 216564.5

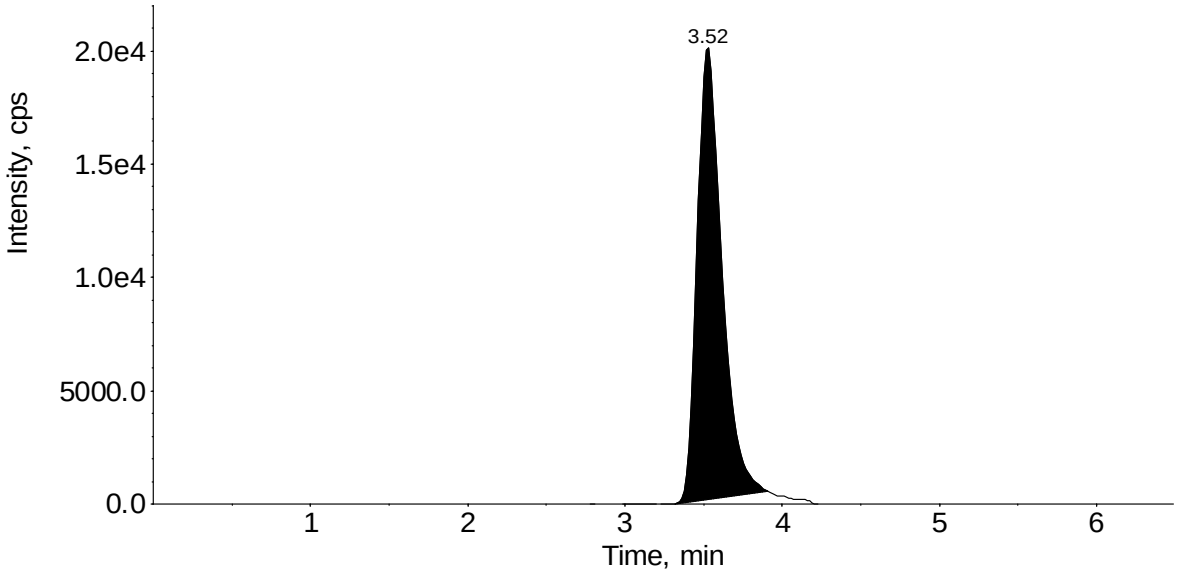


Figure 3 Calibration Curve – Chlorothalonil

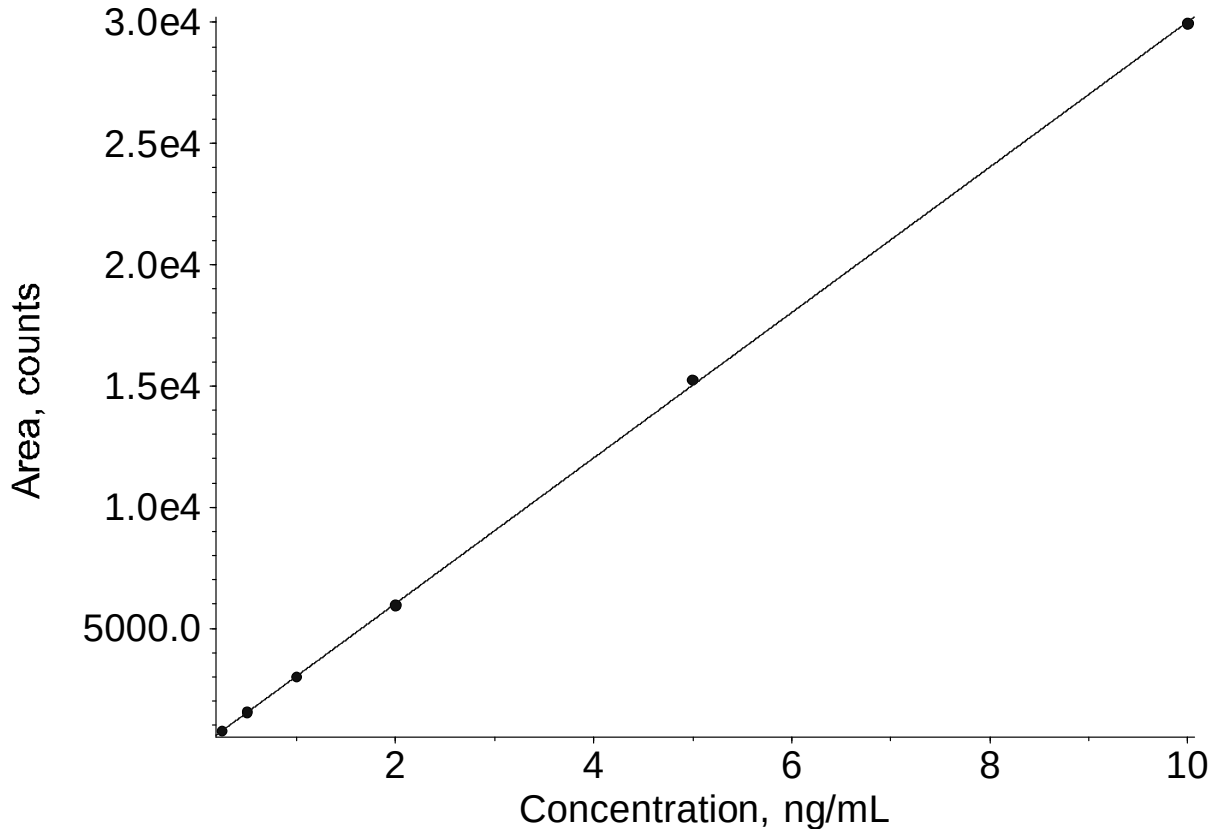


Figure 4 Calibration Curve – R182281

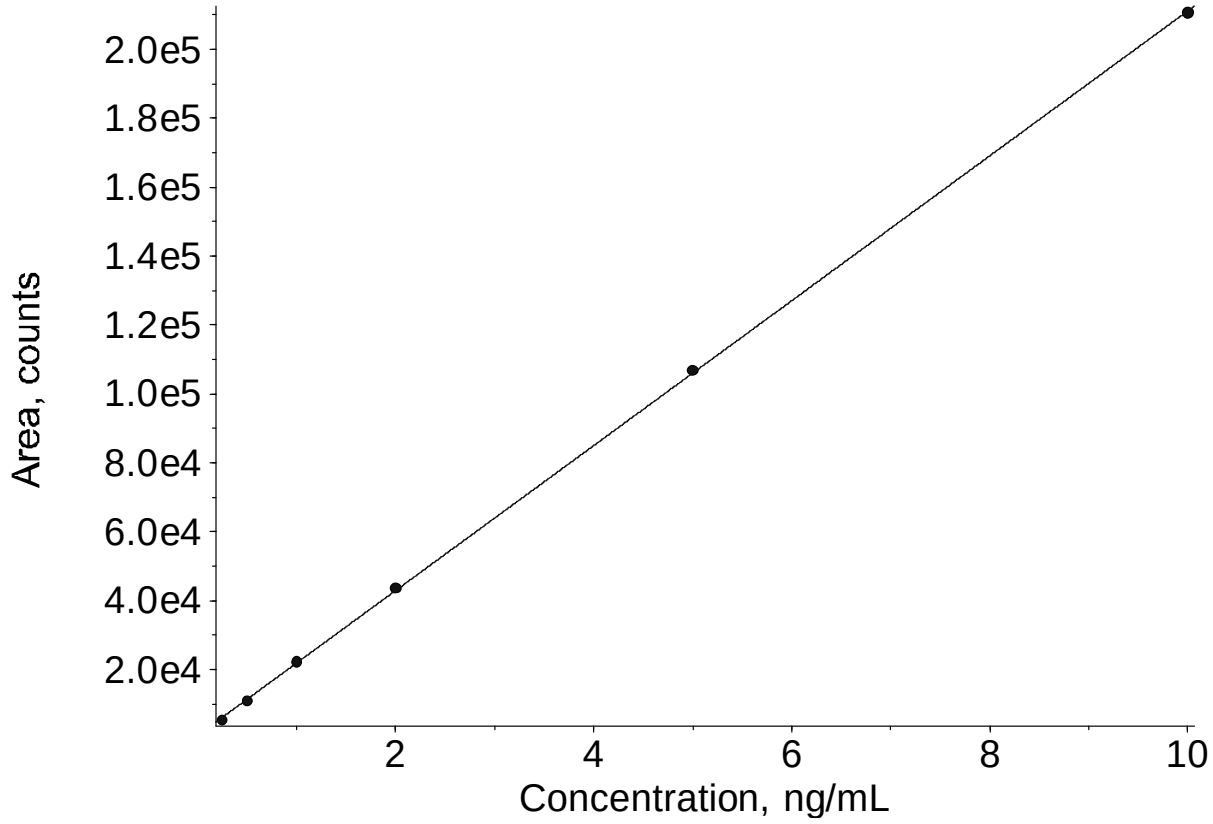
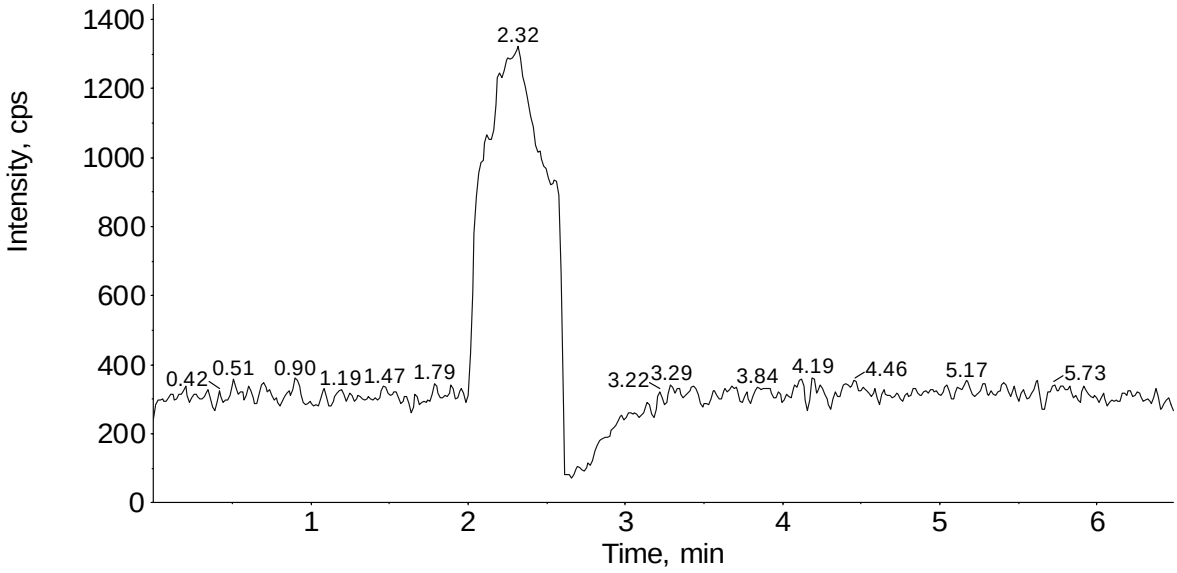


FIGURE 5 Chromatograms - Chlorothalonil in Soybean Seed

Soybean seed control, 0 ppb of Chlorothalonil found, <0.01 ppb determined



Soybean seed control fortified with 9.95 ppb, 10.3 ppb of Chlorothalonil determined, 104% recovery

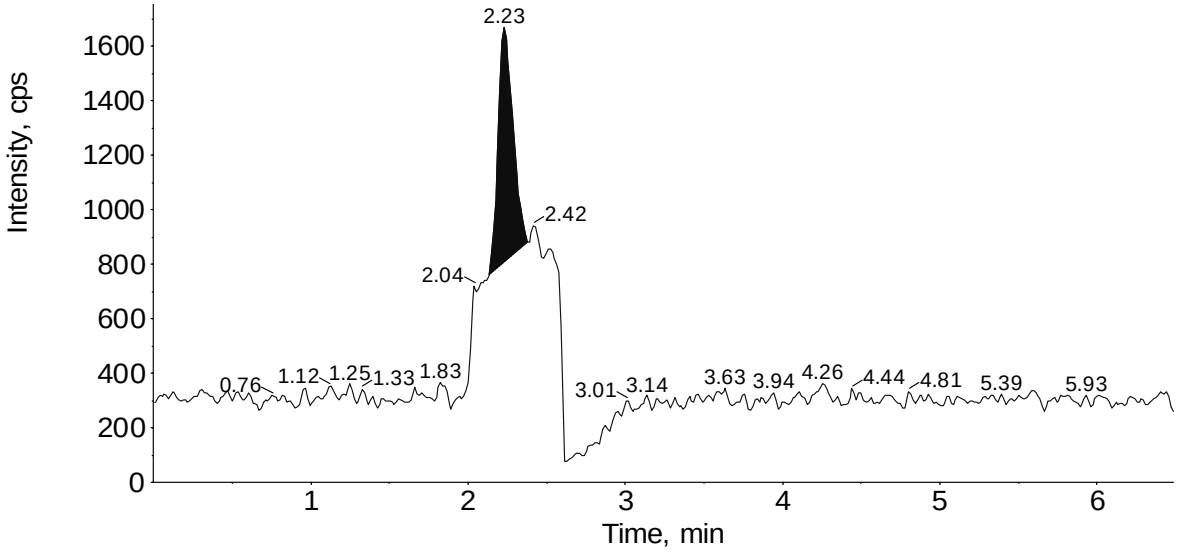


FIGURE 5 Chromatograms - Chlorothalonil in Soybean Seed (Continued)

Soybean seed control fortified with XXX ppb, XX ppb of Chlorothalonil determined, XXX% recovery

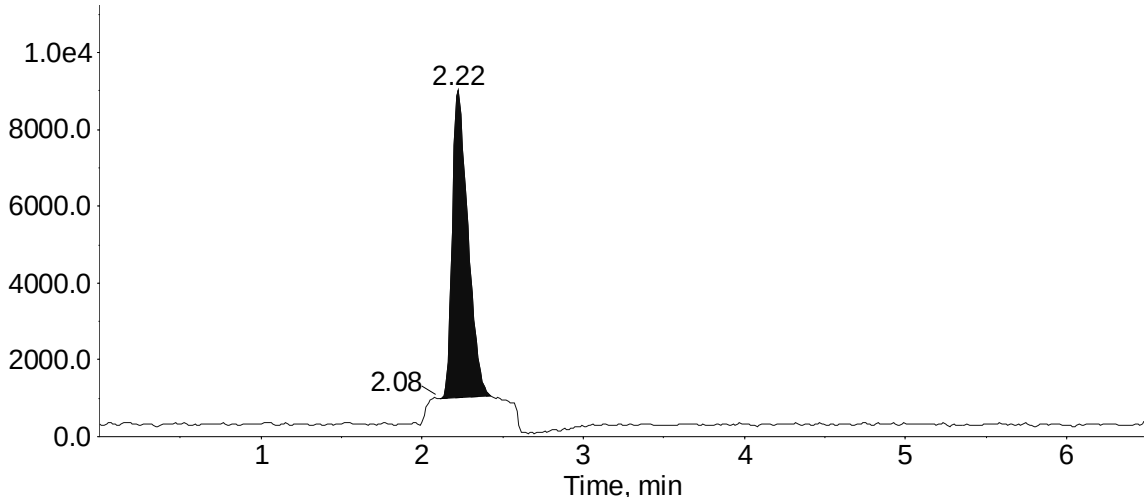
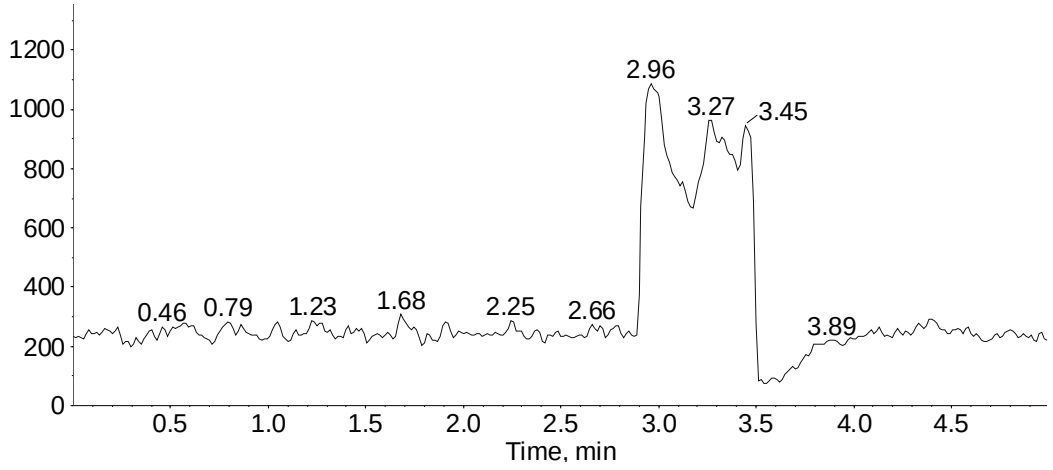


FIGURE 6 Chromatograms - Chlorothalonil in Soybean Forage

Soybean Forage control, 0 ppb of Chlorothalonil found, <0.01 ppb determined



Soybean Forage control fortified with 10.0 ppb, 9.73 ppb of Chlorothalonil determined, 97.3% recovery

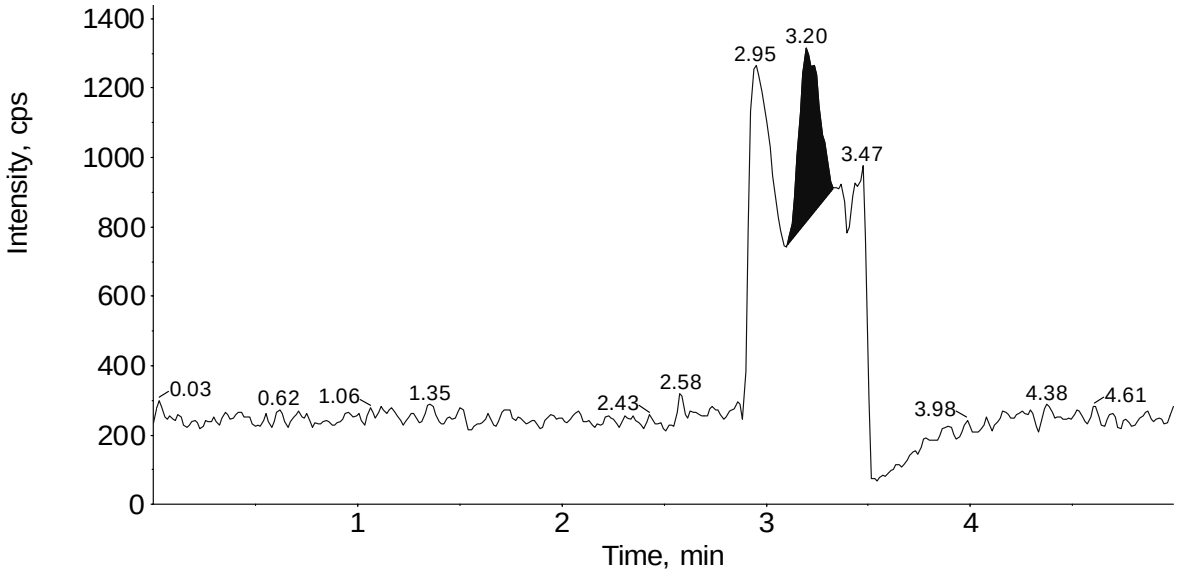


FIGURE 6 Chromatograms - Chlorothalonil in Soybean Forage (Continued)

Soybean Forage control fortified with 105263 ppb, 105343 ppb of Chlorothalonil determined, 100% recovery

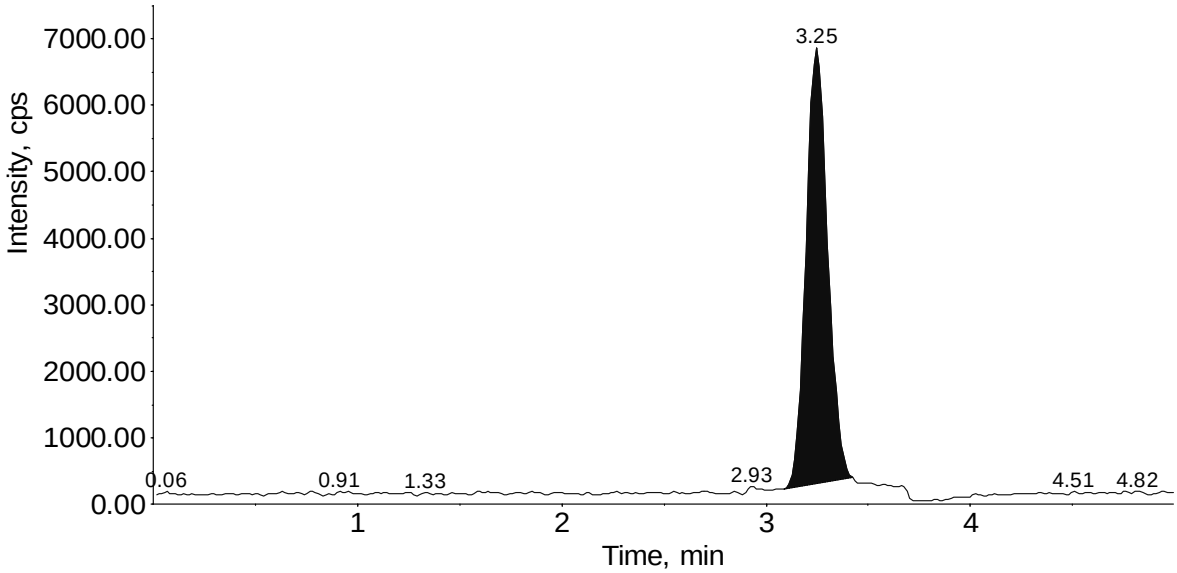
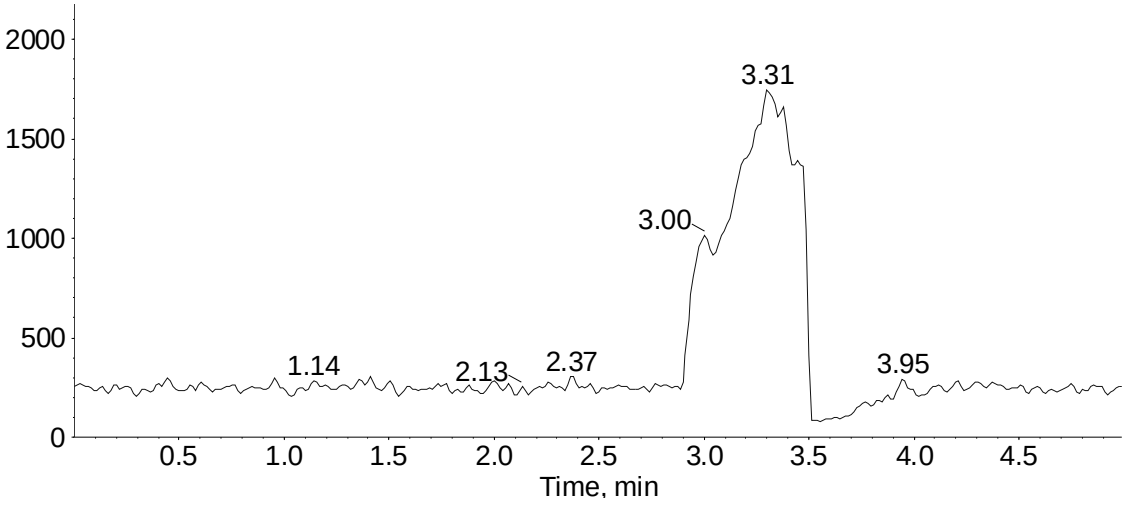


FIGURE 7 Chromatograms - Chlorothalonil in Soybean Hay

Soybean Hay control, 0 ppb of Chlorothalonil found, <0.01 ppb determined



Soybean Hay control fortified with 50.0 ppb, 49.1 ppb of Chlorothalonil determined, 98.2% recovery

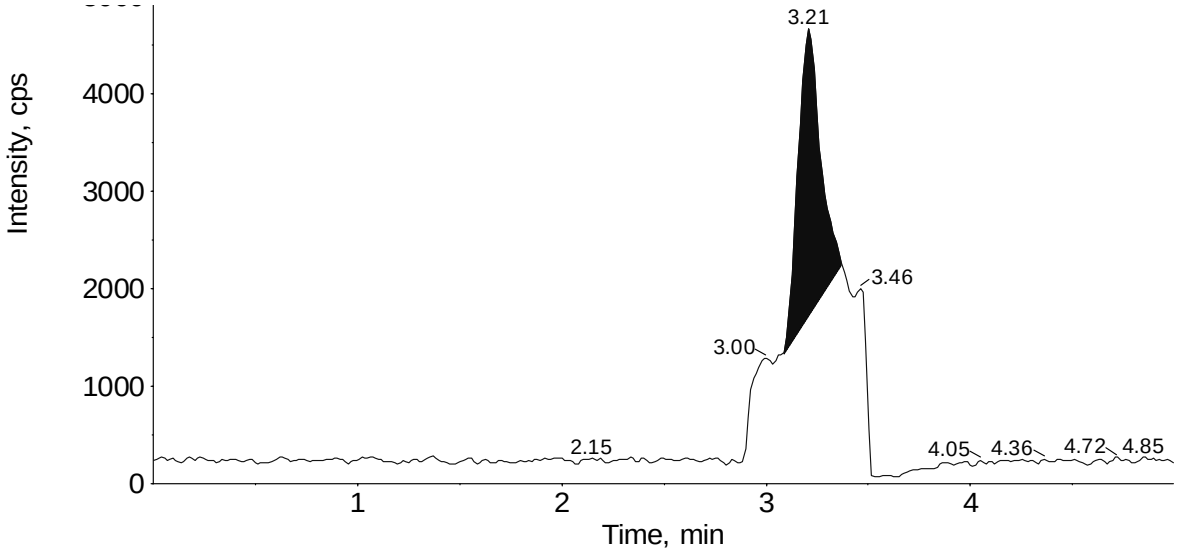


FIGURE 7 Chromatograms - Chlorothalonil in Soybean Hay (Continued)

Soybean Hay control fortified with 504 ppb, 500 ppb of Chlorothalonil determined, 99.2% recovery

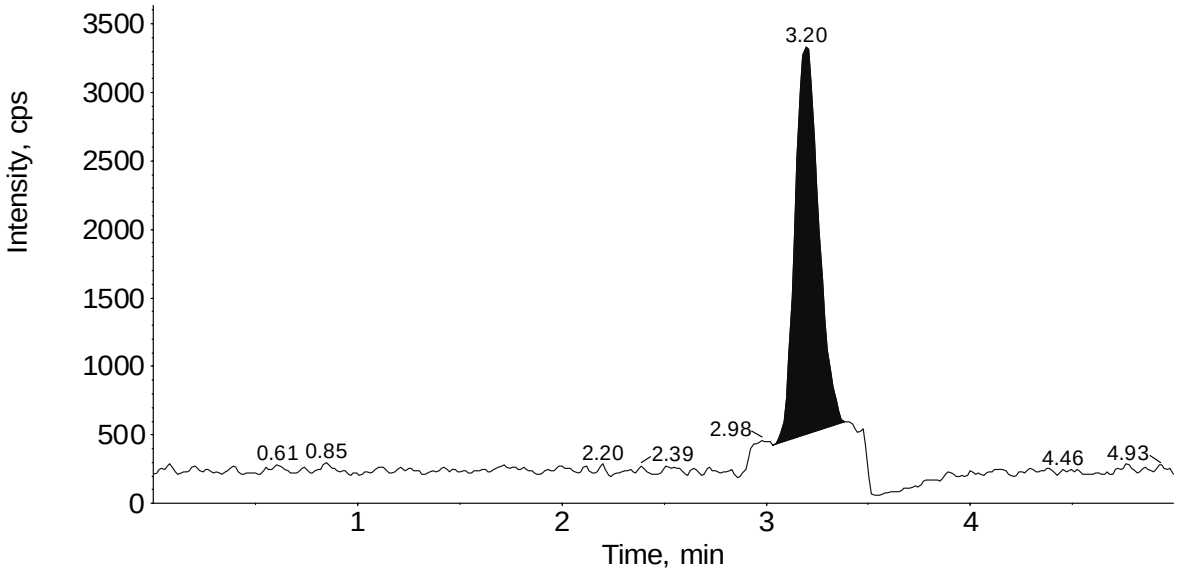
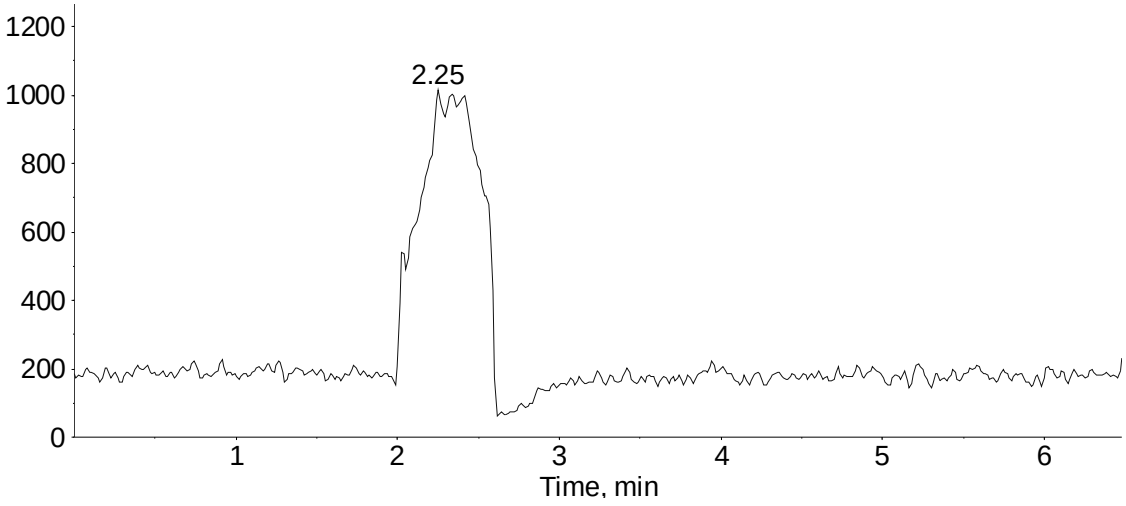


FIGURE 8 Chromatograms - Chlorothalonil in Soybean Meal

Soybean Meal control, 0 ppb of Chlorothalonil found, <0.01 ppb determined



Soybean Meal control fortified with 50.0 ppb, 51.2 ppb of Chlorothalonil determined, 102% recovery

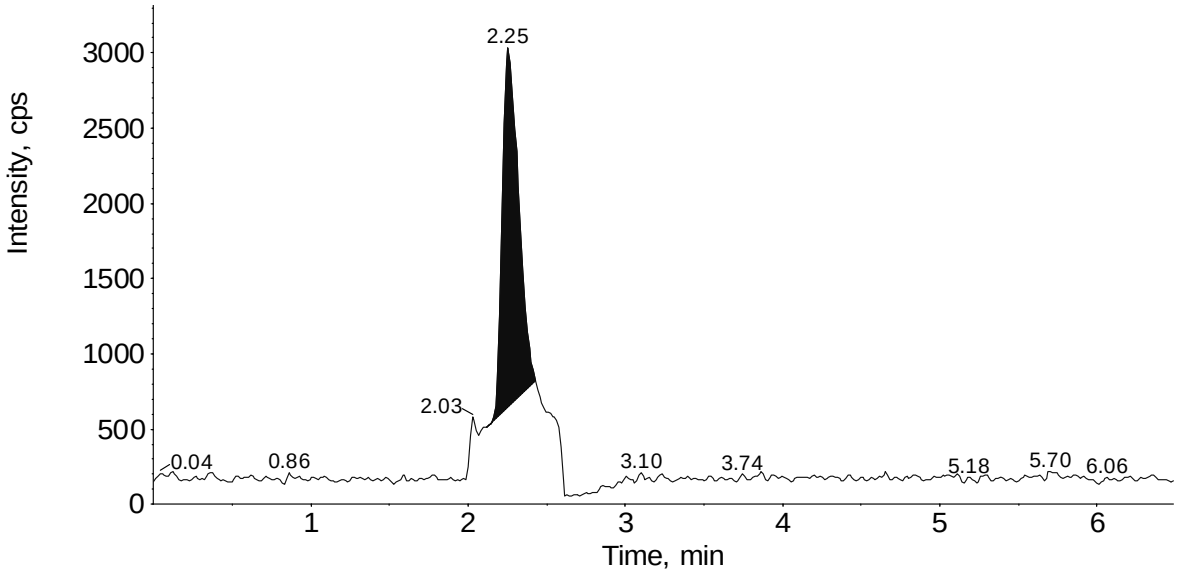


FIGURE 8 Chromatograms - Chlorothalonil in Soybean Meal (Continued)

Soybean Meal control fortified with 498 ppb, 502 ppb of Chlorothalonil determined, 101% recovery

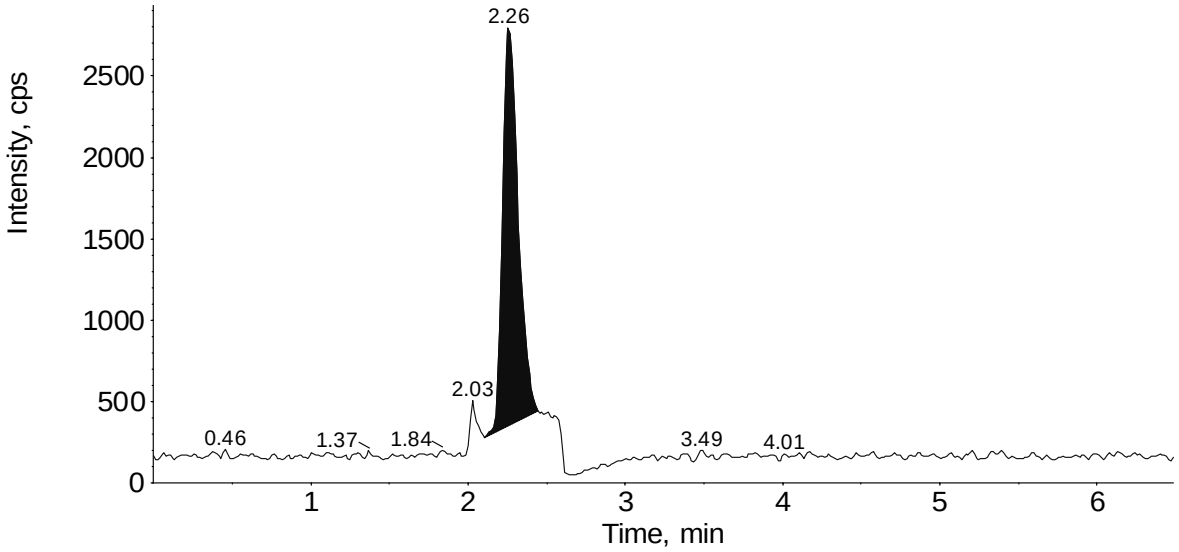
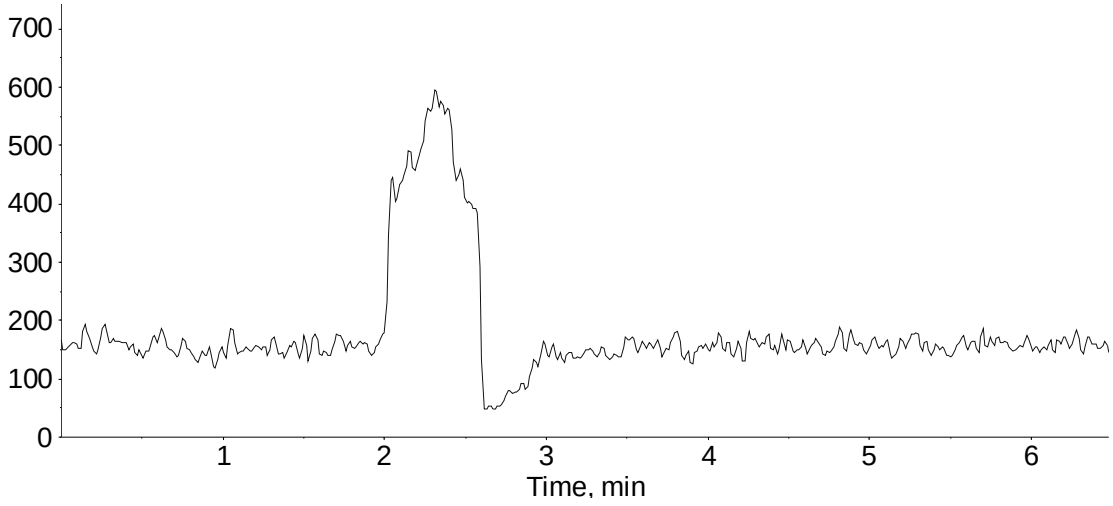


FIGURE 9 Chromatograms - Chlorothalonil in Soybean Hulls

Soybean Hulls control, 0 ppb of Chlorothalonil found, <0.01 ppb determined



Soybean Hulls control fortified with 10.0 ppb, 10.3 ppb of Chlorothalonil determined, 103% recovery

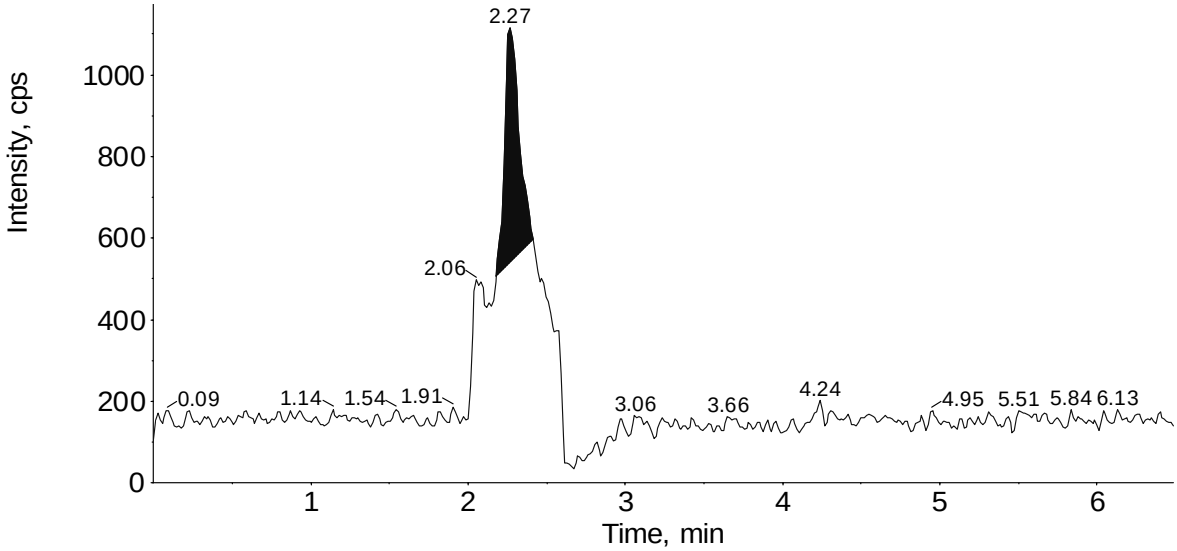


FIGURE 9 Chromatograms - Chlorothalonil in Soybean Hulls (Continued)

Soybean Hulls control fortified with 5195 ppb, 4753 ppb of Chlorothalonil determined, 91.5% recovery

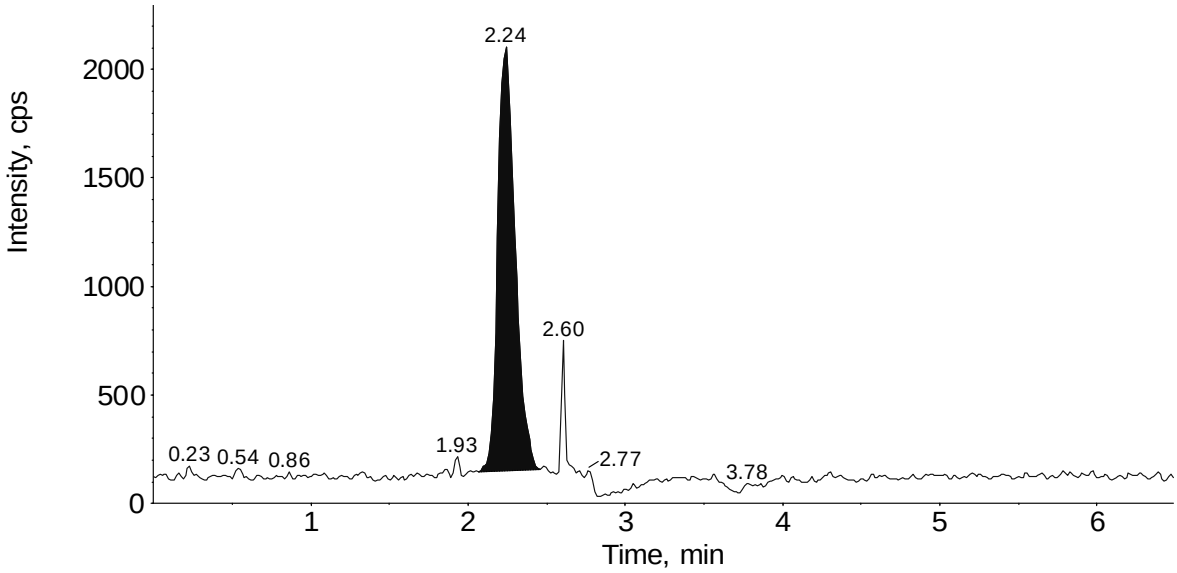
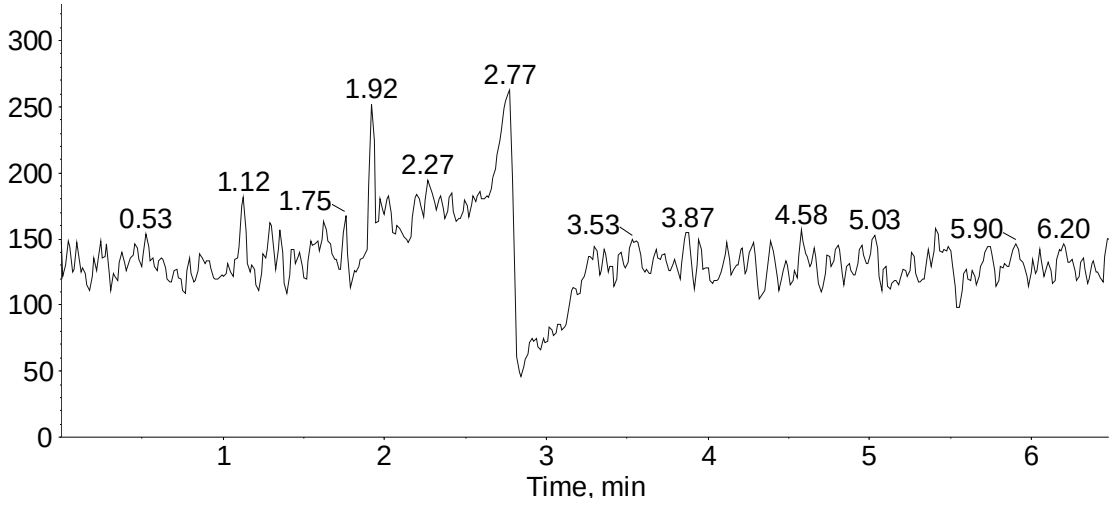


FIGURE 10 Chromatograms - Chlorothalonil in Soybean Oil

Soybean Oil control, 0 ppb of Chlorothalonil found, <0.01 ppb determined



Soybean Oil control fortified with 10.0 ppb, 7.71 ppb of Chlorothalonil determined, 77.1% recovery

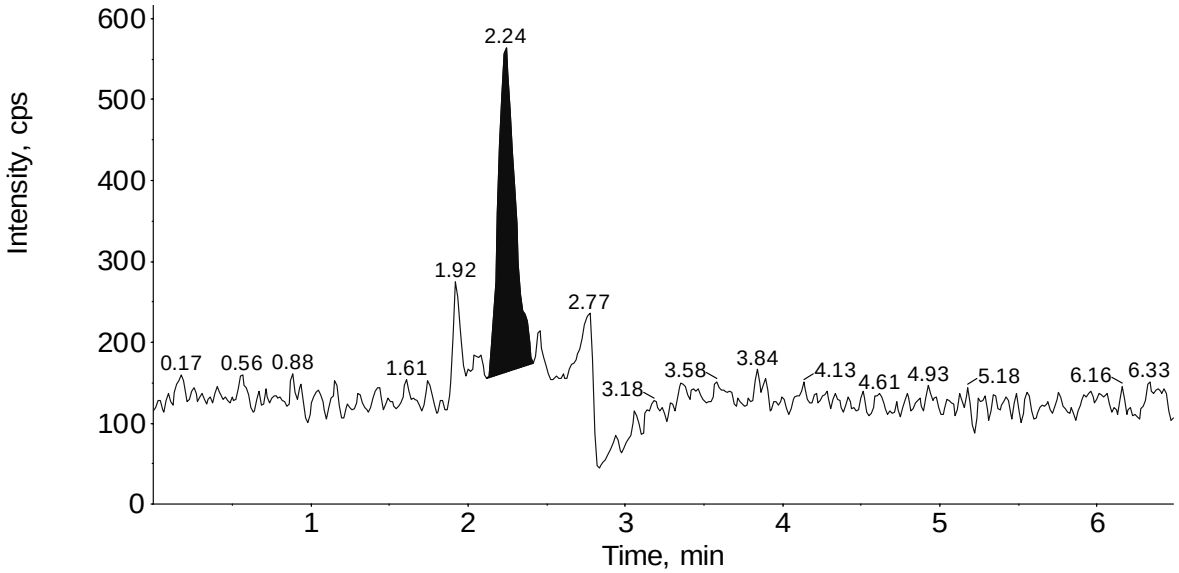


FIGURE 10 Chromatograms - Chlorothalonil in Soybean Oil

Soybean Oil control fortified with 100 ppb, 89.7 ppb of Chlorothalonil determined, 89.7% recovery

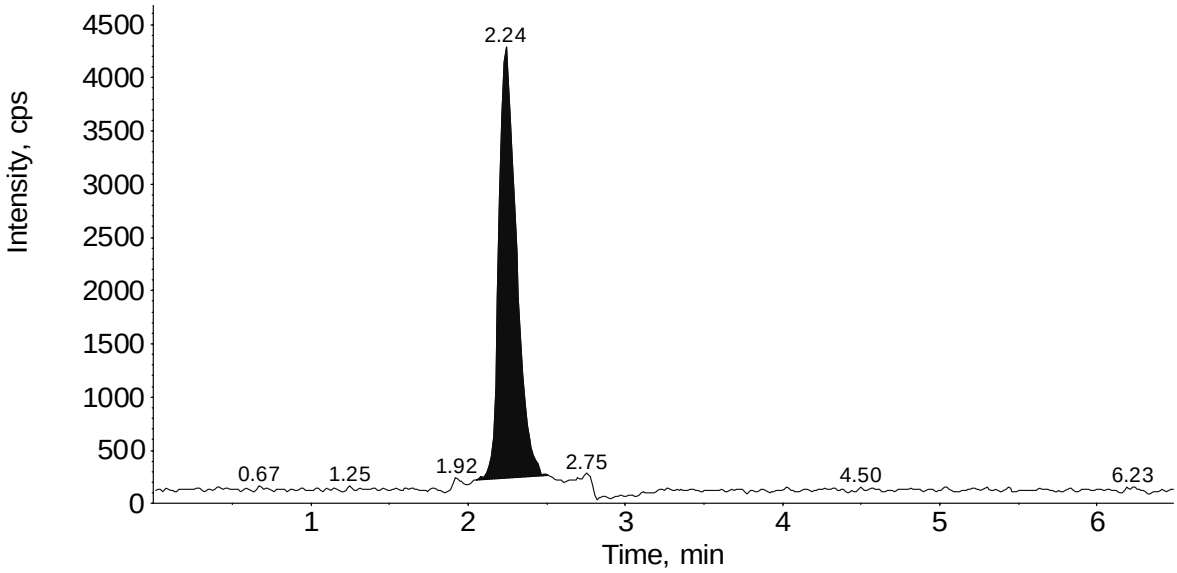
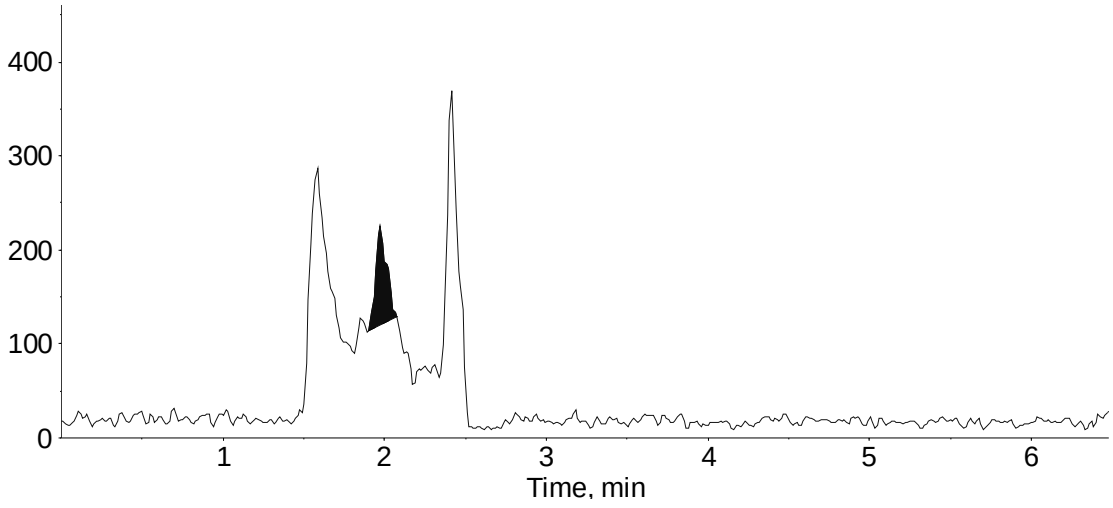


FIGURE 11 Chromatograms - Chlorothalonil in Soybean Aspirated Grain Fractions

Soybean Aspirated Grain Fractions control, 0 ppb of Chlorothalonil found, <0.01 ppb determined



Soybean Aspirated Grain Fractions control fortified with 10.0 ppb, 9.98 ppb of Chlorothalonil determined, 99.8% recovery

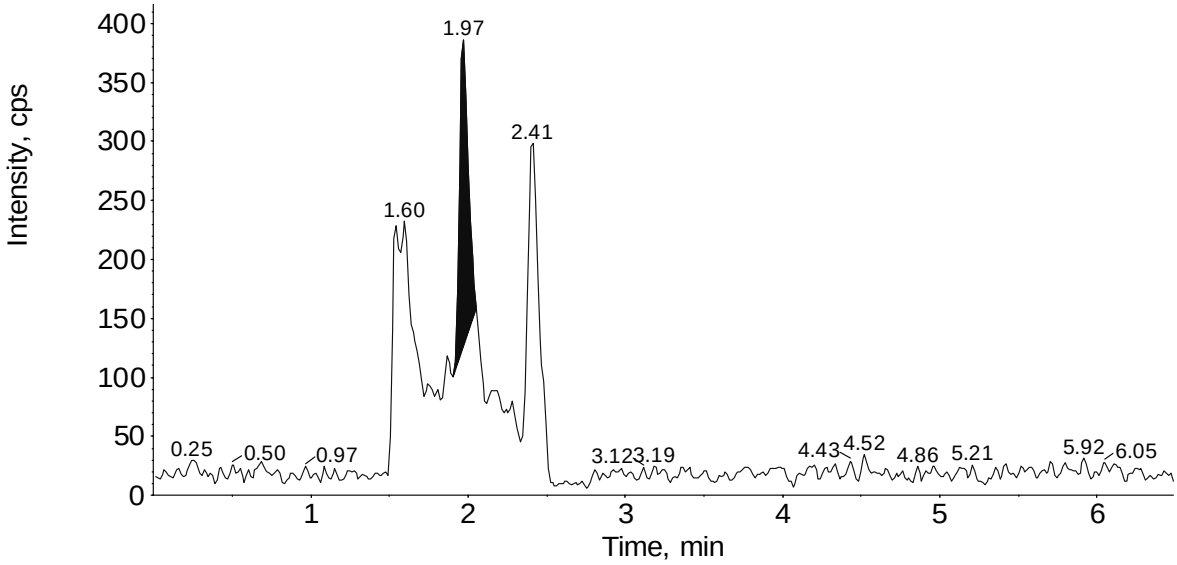


FIGURE 11 Chromatograms - Chlorothalonil in Soybean Aspirated Grain Fractions (Continued)

Soybean Aspirated Grain Fractions control fortified with 97.6 ppb, 97.9 ppb of Chlorothalonil determined, 100% recovery

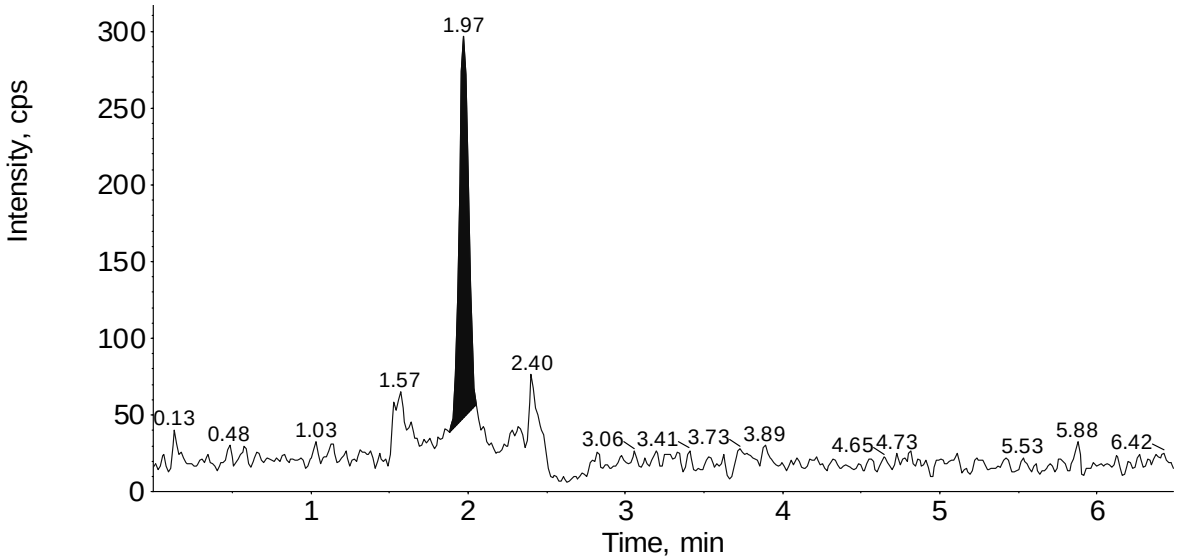
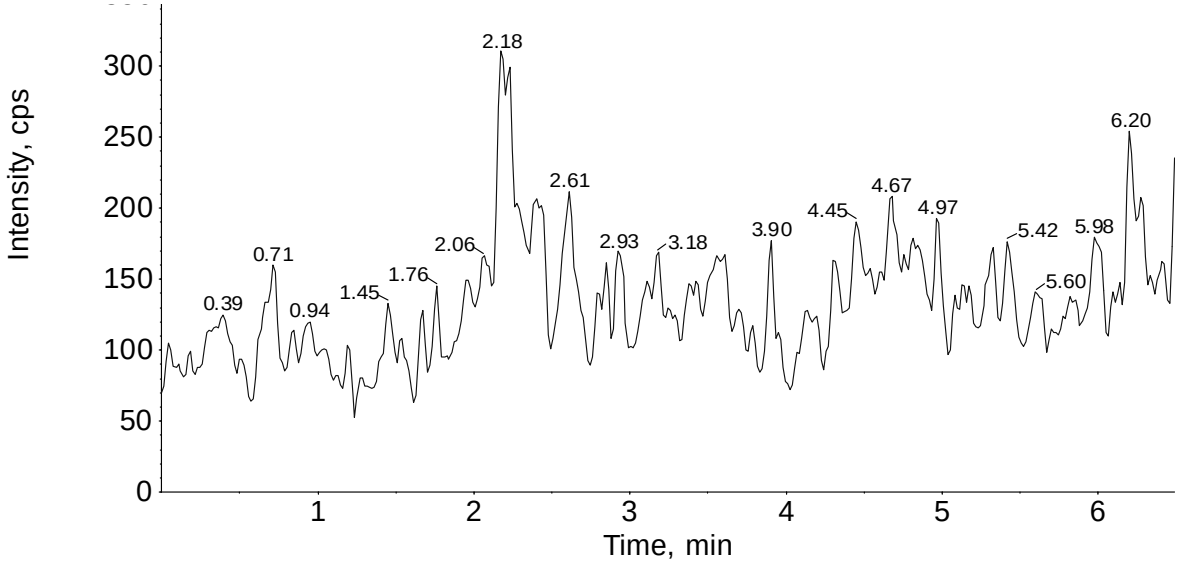


FIGURE 12 Chromatograms - R182281 in Soybean Seed

Soybean seed control, 0 ppb of R182281 found, <0.01 ppb determined



Soybean Seed control fortified with 9.95 ppb, 7.91 ppb of R182281 determined, 79.5% Recovery

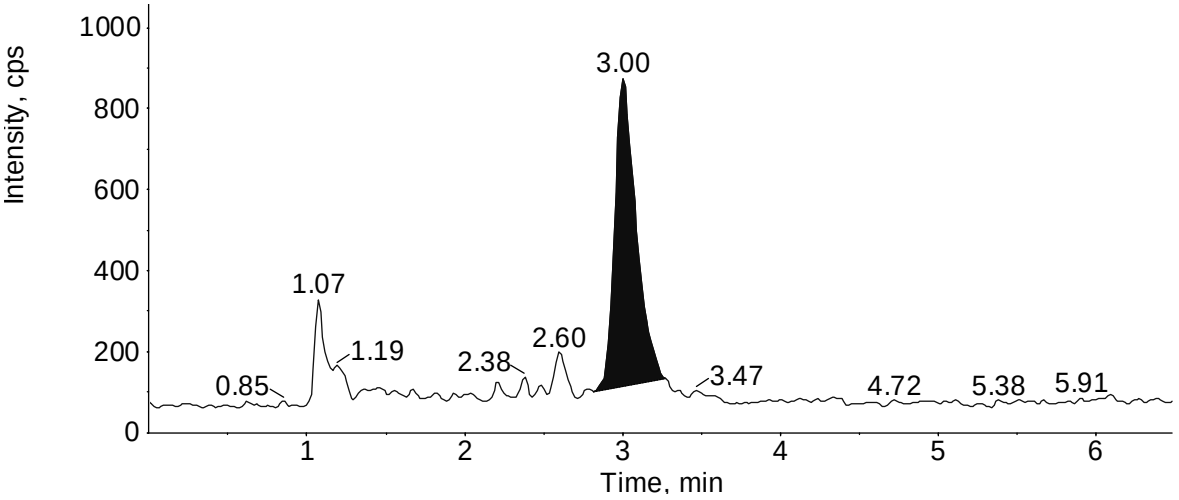


FIGURE 12 Chromatograms - R182281 in Soybean Seed

Soybean seed control fortified with 100 ppb, 82.7 ppb of R182281 determined, 82.7% recovery

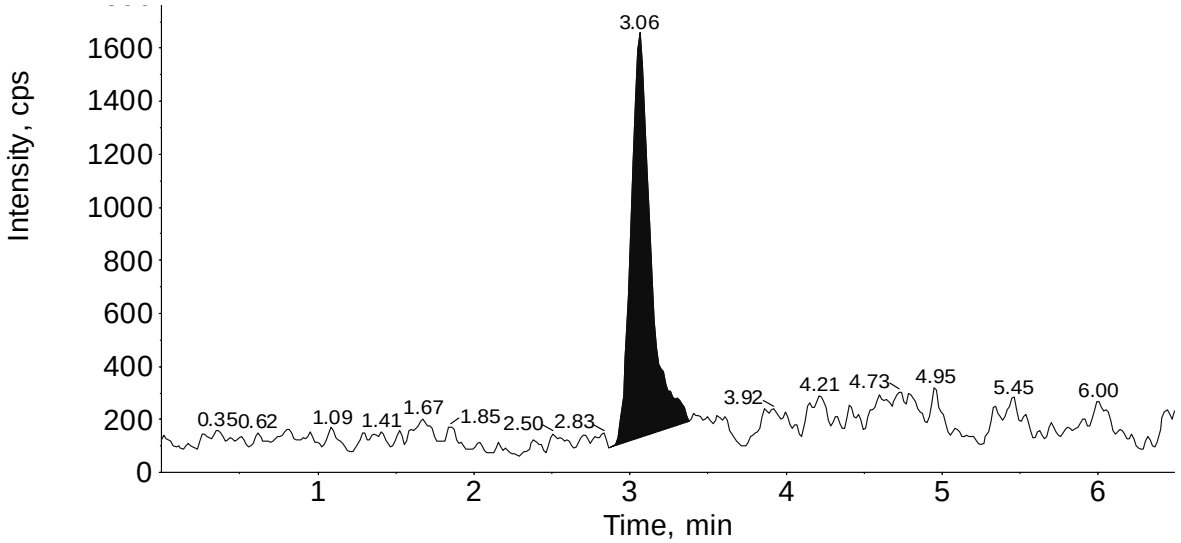
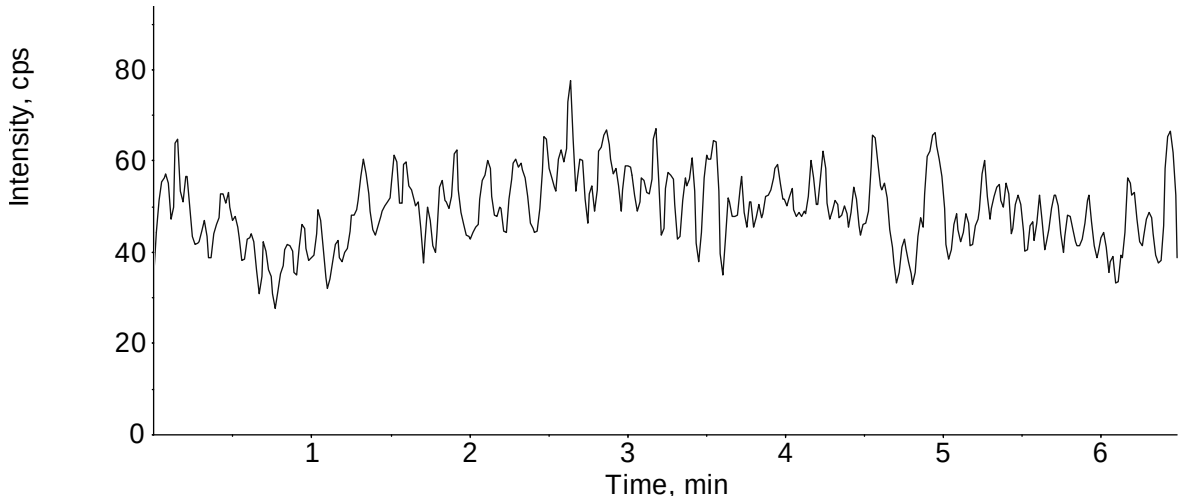


FIGURE 13 Chromatograms - R182281 in Soybean Forage

Soybean Forage control, 0 ppb of R182281 found, <0.01 ppb determined



Soybean Forage control fortified with 10.0 ppb, 10.0 ppb of R182281 determined, 100% recovery

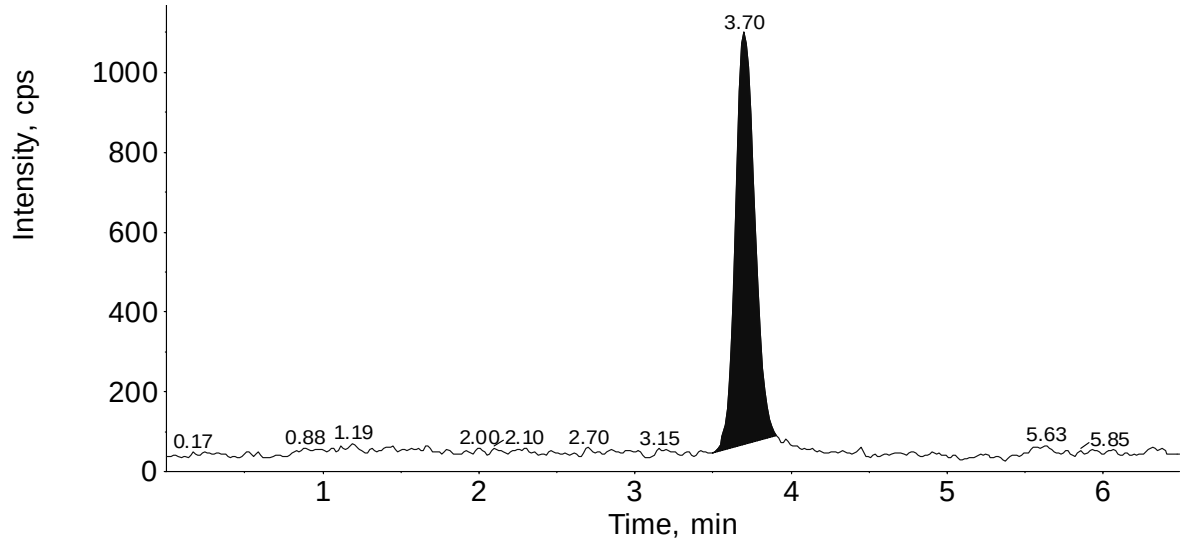


FIGURE 13 Chromatograms - R182281 in Soybean Forage (Continued)

Soybean Forage control fortified with 995 ppb, 1027 ppb of R182281 determined, 103% recovery

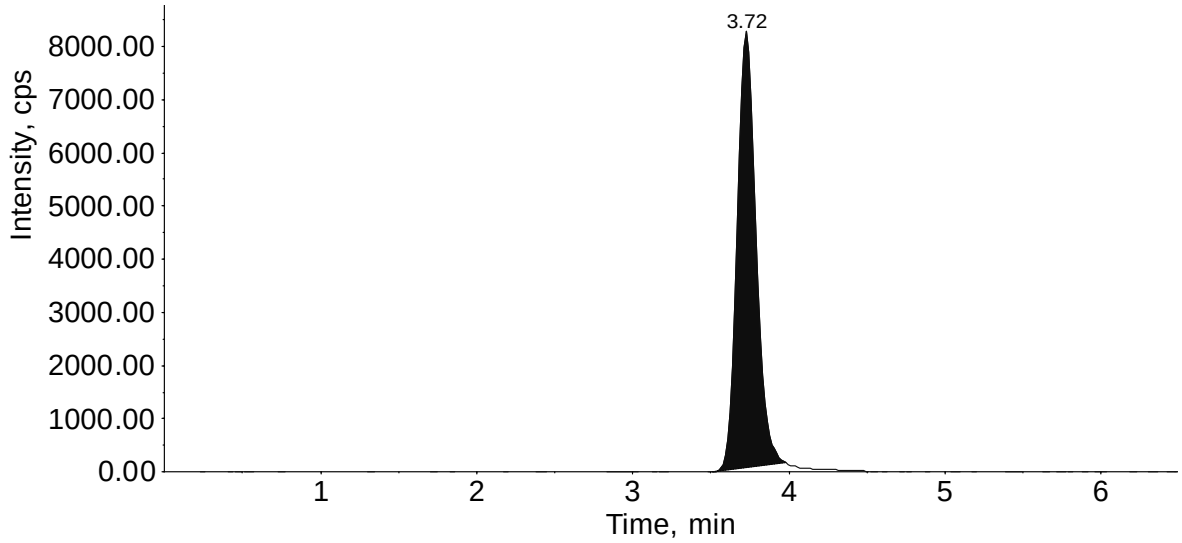
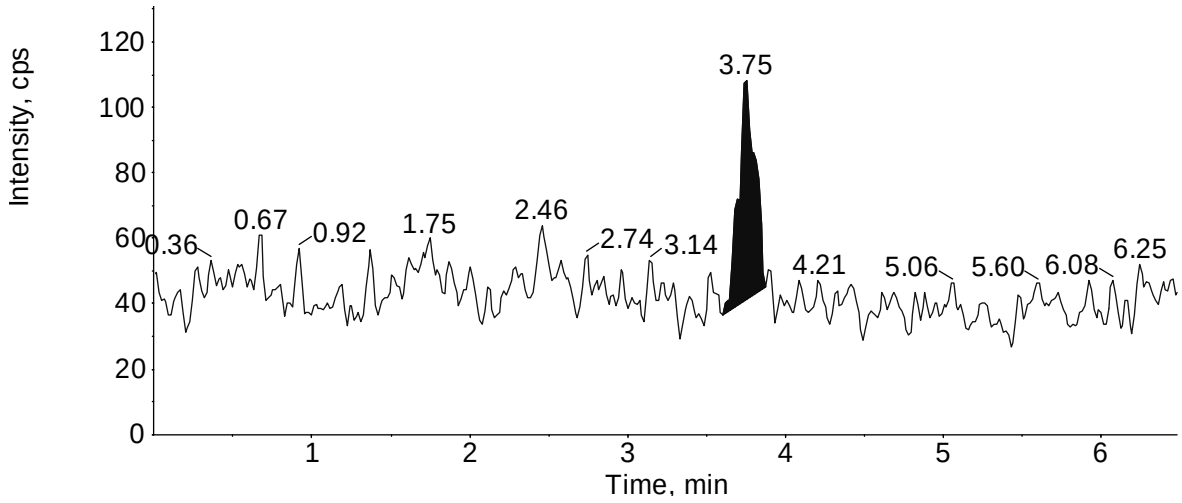


FIGURE 14 Chromatograms - R182281 in Soybean Hay

Soybean Hay control, 0 ppb of R182281 found, <0.01 ppb determined



Soybean Hay control fortified with 10.0 ppb, 10.0 ppb of R182281 determined, 100% recovery

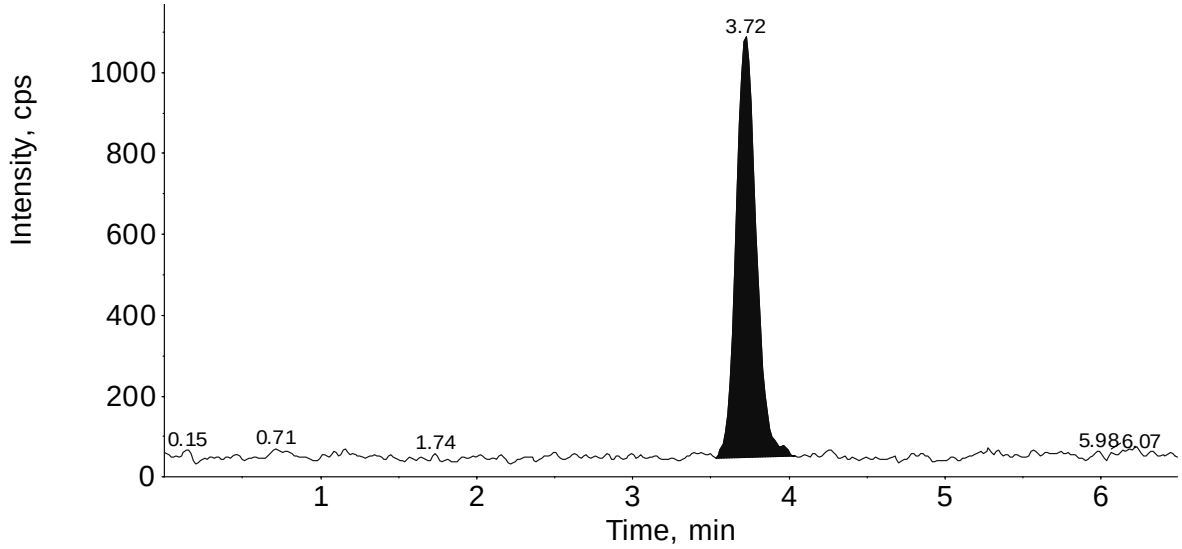


FIGURE 14 Chromatograms - R182281 in Soybean Hay (Continued)

Soybean Hay control fortified with 99.6 ppb, 100 ppb of R182281 determined, 100% recovery

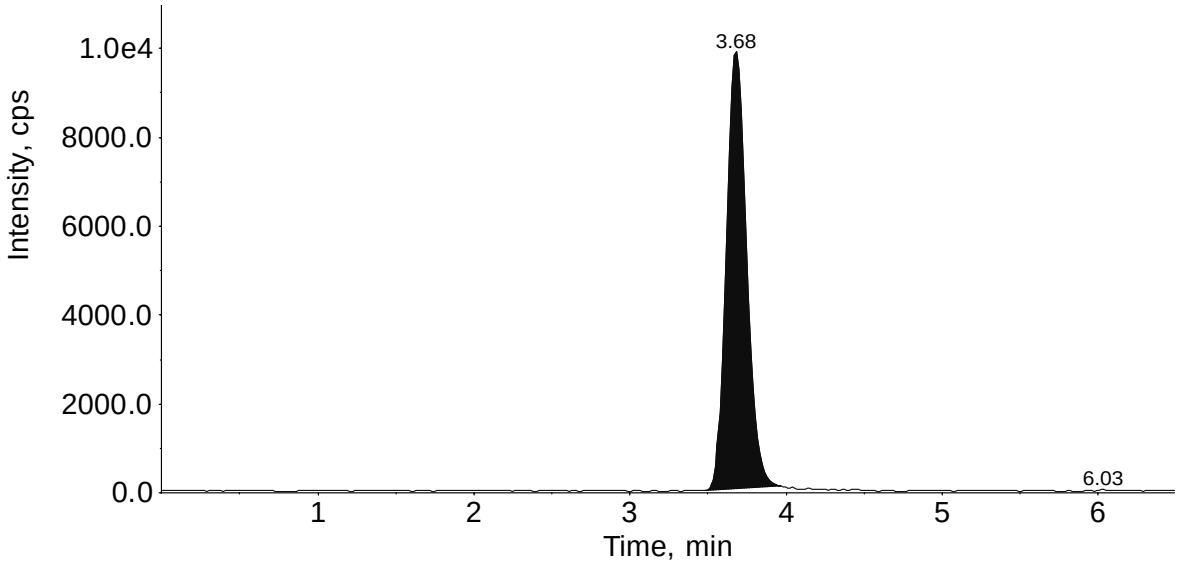
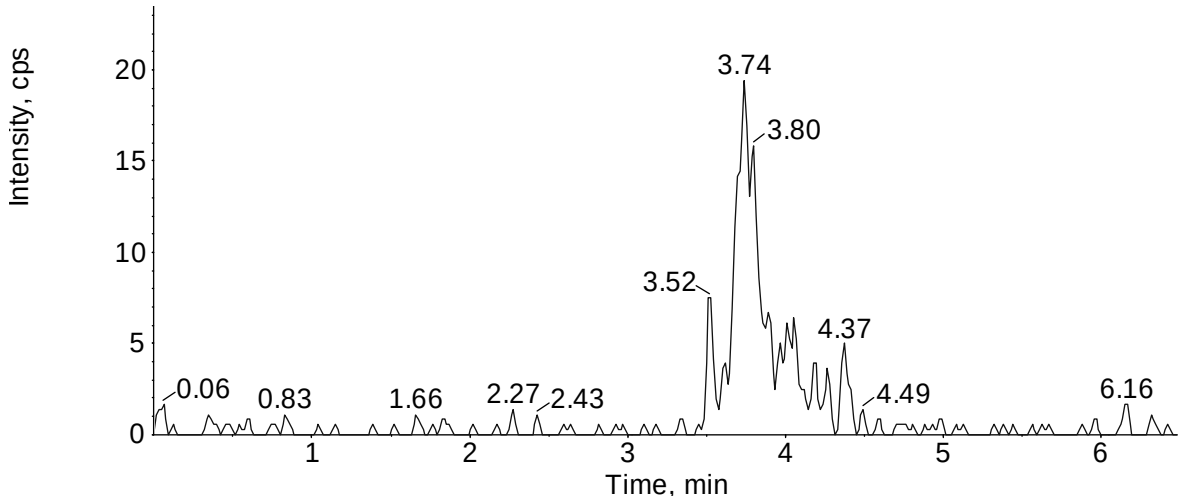


FIGURE 15 Chromatograms - R182281 in Soybean Meal

Soybean Meal control, 0 ppb of R182281 found, <0.01 ppb determined



Soybean Meal control fortified with 9.95 ppb, 10.2 ppb of R182281 determined, 103% recovery

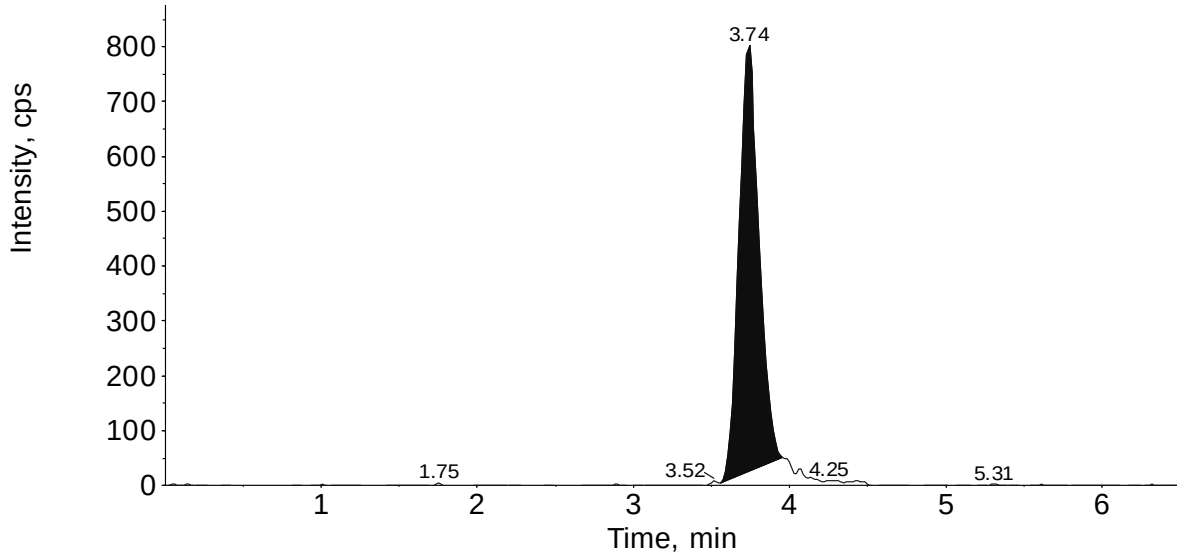


FIGURE 15 Chromatograms - R182281 in Soybean Meal (Continued)

Soybean Meal control fortified with 100 ppb, 102 ppb of R182281 determined, 102% recovery

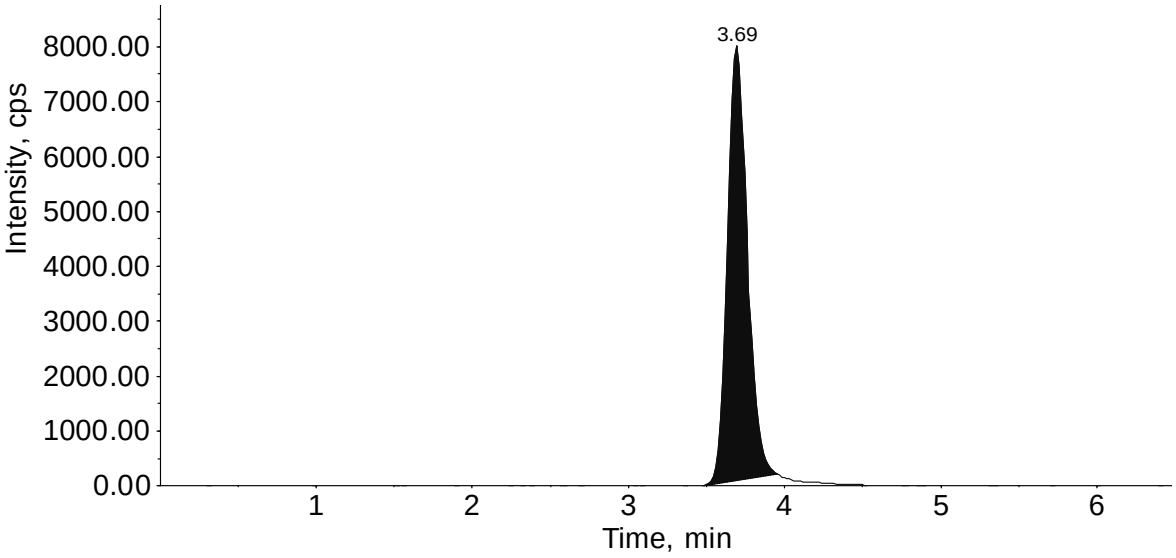
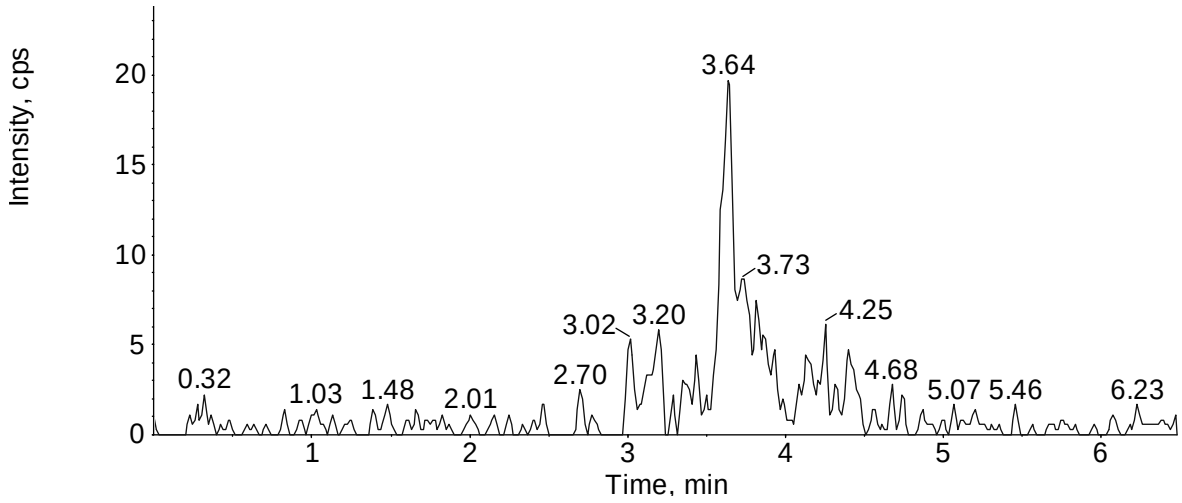


FIGURE 16 Chromatograms - R182281 in Soybean Hulls

Soybean Hulls control, 0 ppb of R182281 found, <0.01 ppb determined



Soybean Hulls control fortified with 9.95 ppb, 10.8 ppb of R182281 determined, 109% recovery

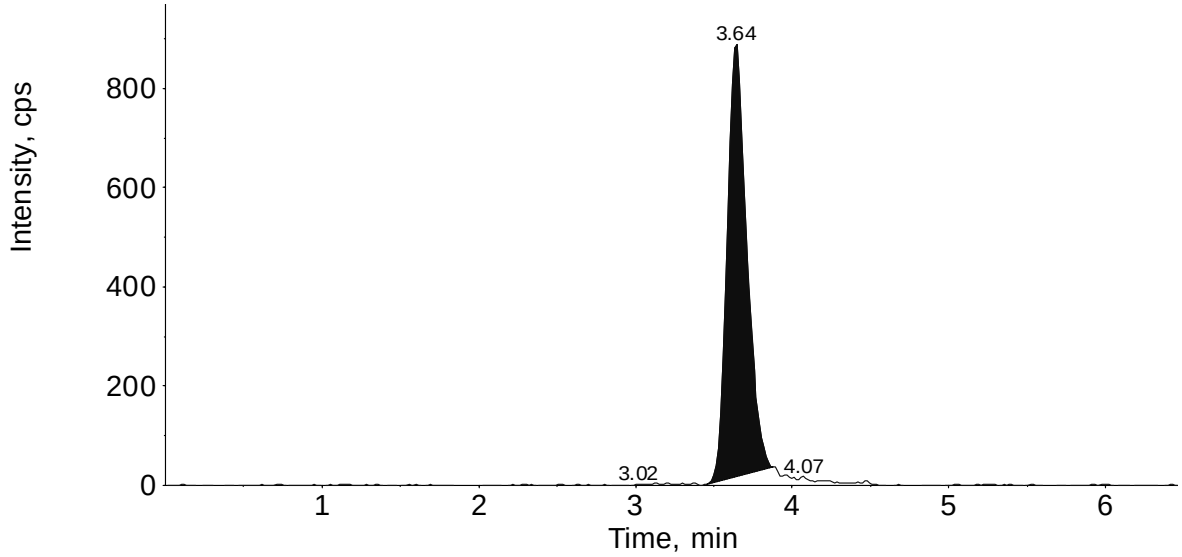


FIGURE 16 Chromatograms - R182281 in Soybean Hulls (Continued)

Soybean Hulls control fortified with 99.7 ppb, 107 ppb of R182281 determined, 107% recovery

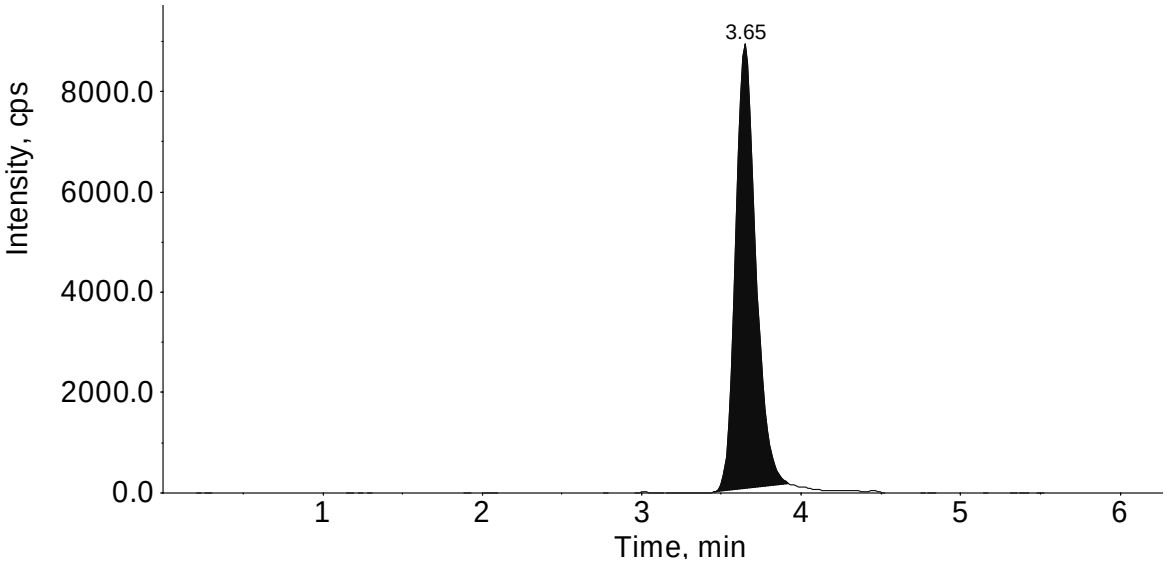
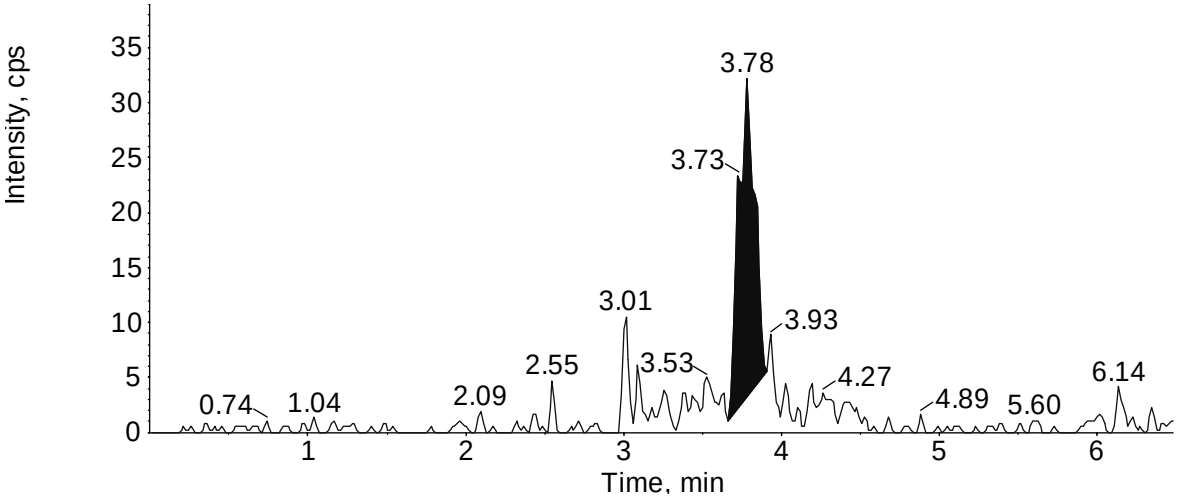


FIGURE 17 Chromatograms - R182281 in Soybean Oil

Soybean Oil control, 0 ppb of R182281 found, <0.01 ppb determined



Soybean Oil control fortified with 10.0 ppb, 7.57 ppb of R182281 determined, 75.7% recovery

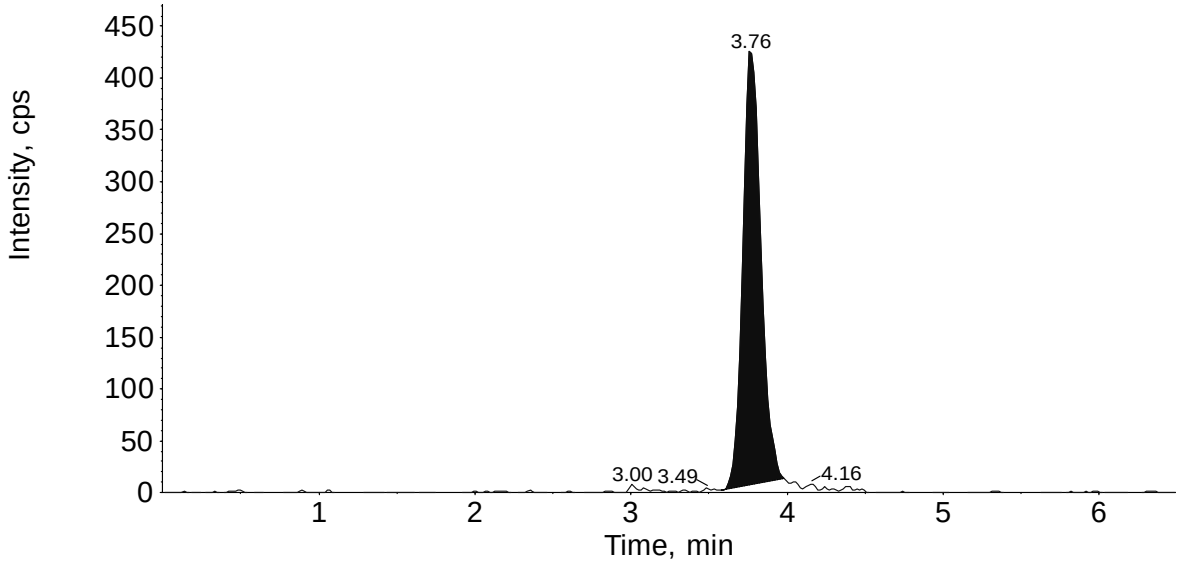


FIGURE 17 Chromatograms - R182281 in Soybean Oil (Continued)

Soybean Oil control fortified with 100 ppb, 81.0 ppb of R182281 determined, 81.0% recovery

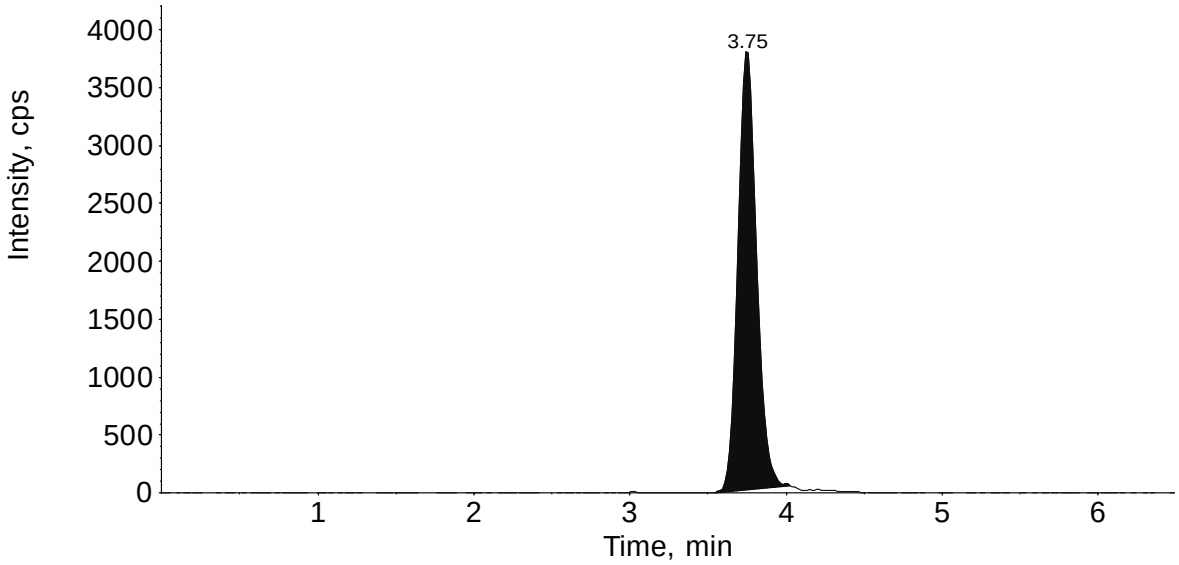
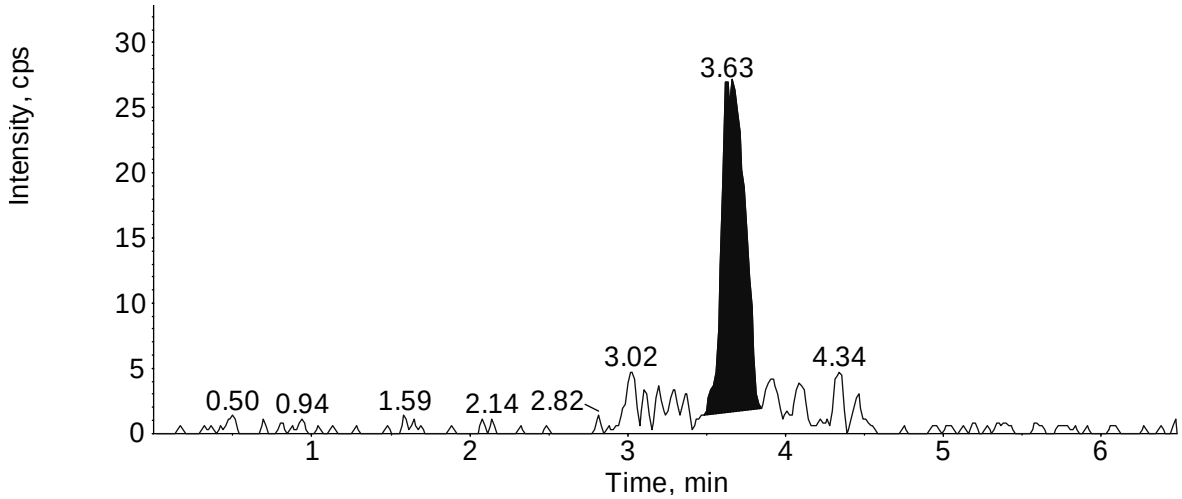


FIGURE 18 Chromatograms - R182281 in Soybean Aspirated Grain Fractions

Soybean Aspirated Grain Fractions control, 0 ppb of R182281 found, <0.01 ppb determined



Soybean Aspirated Grain Fractions control fortified with 9.62 ppb, 10.4 ppb of R182281 determined, 108% recovery

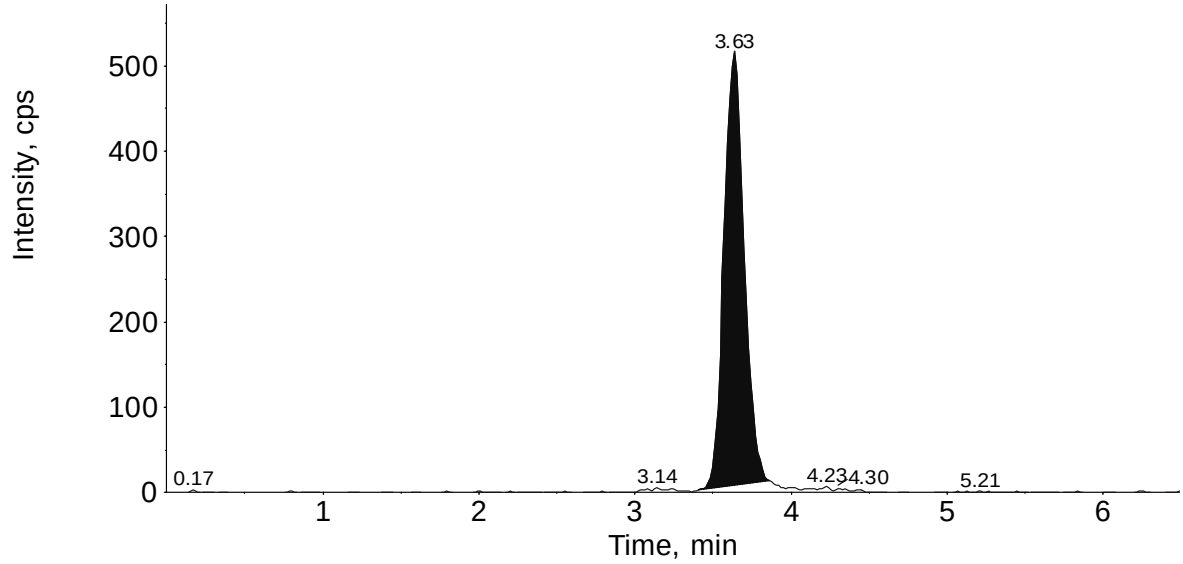
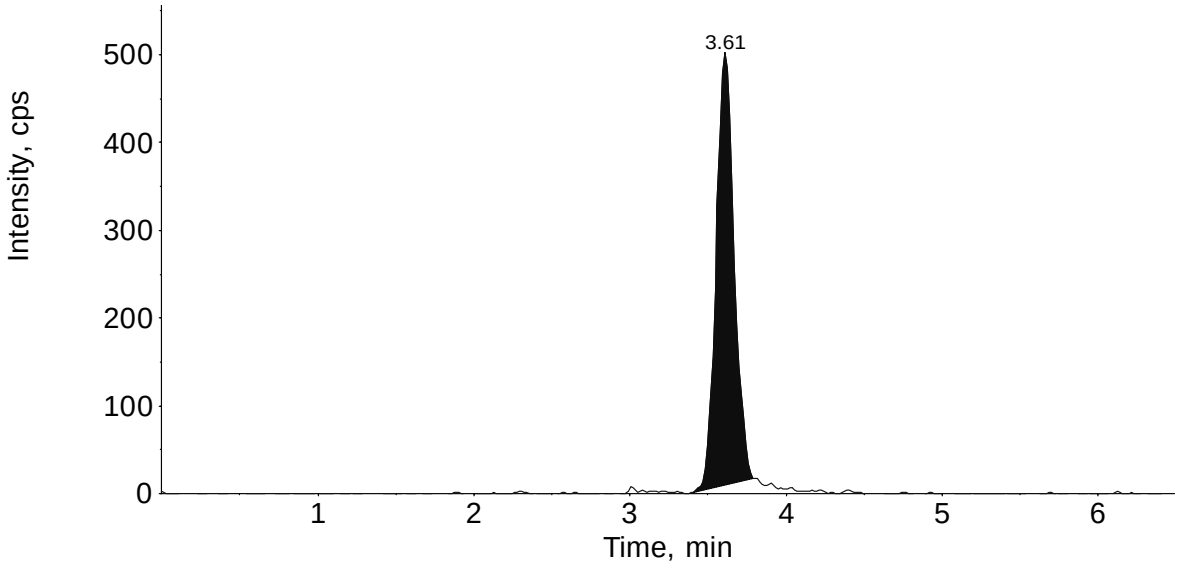


FIGURE 18 Chromatograms - R182281 in Soybean Aspirated Grain Fractions (Continued)

Soybean Aspirated Grain Fractions control fortified with 97.6 ppb, 89.5 ppb of R182281 determined, 91.7% recovery



APPENDIX 4 Method Trial Data

Chlorothalonil Recovery Data Obtained During Method Trials

Matrix	Fortification (ppb)	Sample Concentration (ppb)	Percent Recovery (%)
Soybean Seed	9.95	10.3	104
	10.0	9.82	98.2
	9.95	9.70	97.5
	99.8	112	112
	99.6	111	111
	100	107	107
	2082	1983	95.2
	2068	2197	106
	2082	2066	99.2
Soybean Forage	10.0	11.2	112
	9.97	8.85	88.8
	10.0	9.73	97.3
	99.0	89.1	90.0
	100	91.1	91.1
	99.2	92.8	93.5
	105473	116677	111
	105263	105343	100
	105263	99404	94.4
Soybean Hay	50.3	42.9	85.3
	50.0	42.9	85.8
	50.0	49.1	98.2
	504	500	99.2
	500	478	95.6
	498	458	92.0
	316418	352876	112
	317365	323832	102
	317049	344247	109
	49.8	47.3	95.0
Soybean Meal	49.8	48.6	97.6
	50.0	51.2	102
	498	502	101
	500	442	88.4
	498	484	97.2

APPENDIX 4 Method Trial Data (Continued)

Chlorothalonil Recovery Data Obtained During Method Trials (Continued)

Matrix	Fortification (ppb)	Sample Concentration (ppb)	Percent Recovery (%)
Soybean Hulls	10.0	10.3	103
	9.99	11.4	114
	9.95	10.8	109
	100	103	103
	99.7	100	100
	99.5	96.8	97.3
	5195	4597	88.5
	5179	4428	85.5
	5195	4753	91.5
Soybean Oil	10.0	7.05	70.5
	10.0	7.71	77.1
	10.0	7.03	70.3
	100	89.7	89.7
	100	84.0	84.0
	100	85.4	85.4
Soybean Aspirated Grain Fractions	9.62	10.1	105
	9.76	10.5	108
	10.0	9.98	99.8
	97.6	97.9	100
	98.5	102	104
	98.5	107	109
	204926	218759	107
	202927	209522	103
	203922	203412	100
		Mean	**97.8**
		Standard Deviation	**9.90**
		RSD %	**10.1**
		Minimum	**70.3**
		Maximum	**114**
		n	**57**

APPENDIX 4 Method Trial Data (Continued)

R182281 Recovery Data Obtained During Method Trials

Matrix	Fortification (ppb)	Sample Concentration (ppb)	Percent Recovery (%)
Soybean Seed	9.95	7.91	79.5
	10.0	8.03	80.3
	9.95	8.41	84.5
	99.8	80.6	80.8
	99.6	81.4	81.7
	100	82.7	82.7
Soybean Forage	10.0	12.6	126
	9.97	10.1	101
	10.0	10.0	100
	99.0	99.3	100
	100	102	102
	99.2	103	104
	995	1027	103
	993	1115	112
	993	1050	106
Soybean Hay	10.1	10.3	102
	10.0	10.0	100
	10.0	10.5	105
	101	104	103
	99.9	99.4	99.5
	99.6	100	100
	1990	2114	106
	1996	2265	113
	1994	2398	120
Soybean Meal	9.95	9.83	98.8
	9.95	10.2	103
	10.0	9.80	98.0
	99.5	103	104
	100	102	102
	99.5	103	104

APPENDIX 4 Method Trial Data (Continued)

R182281 Recovery Data Obtained During Method Trials (Continued)

Matrix	Fortification (ppb)	Sample Concentration (ppb)	Percent Recovery (%)
Soybean Hulls	10.0	11.6	116
	9.99	11.0	110
	9.95	10.8	109
	100	111	111
	99.7	107	107
	99.5	114	115
	500	457	91.4
	498	453	91.0
	500	450	90.0
Soybean Oil	10.0	7.08	70.8
	10.0	7.74	77.4
	10.0	7.57	75.7
	100	78.8	78.8
	100	81.4	81.4
	100	81.0	81.0
Soybean Aspirated Grain Fractions	9.62	10.4	108
	9.76	10.7	110
	10.0	10.6	106
	97.6	89.5	91.7
	98.5	45.8	46.5
	98.5	98.5	100
	4926	4951	101
	4878	4634	95.0
	4902	5147	105
		Mean	**97.6**
		Standard Deviation	**14.2**
		RSD %	**14.5**
		Minimum	**46.5**
		Maximum	**126**
		n	**54**