



GRM052.01A

**Analytical Method GRM052.01A for the Determination of
Simazine, G28273 and G28279 in Crops. Final
Determination by LC-MS/MS**

Method

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Summary of revisions to previous version

Version Summary of revisions

GRM052.01A New method - Not Applicable.

Author:

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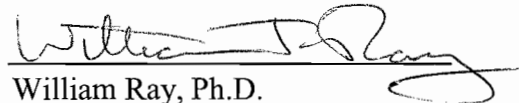


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Abbreviations and symbols

Abbreviation	Definition
A	Acre
a.i.	active ingredient
amt	Amount
amu	atomic mass unit
C	Celsius or Centigrade
CAS	Chemical Abstract Services
CFR	Code of Federal Regulations
cm	Centimeter
DA[#]A	days after application, [#] = 1, 2, 3 etc., if there are multiple applications
EPA	Environmental Protection Agency (U.S.)
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (U.S.)
ft	foot (feet)
g	Gram
gal	Gallon
GC	gas chromatography
GLP	Good Laboratory Practice
GRM	Global Residue Method
ha	Hectare
HPLC	high performance liquid chromatography
i.d.	inside diameter
ID	Identification
in	Inch
IUPAC	International Union of Pure and Applied Chemistry
kg	Kilogram
L	Liter
lb	Pound
LC	liquid chromatography
LC-MS	tandem liquid chromatography/mass spectrometry
LC-MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
m	Meter

Abbreviations and symbols (continued)

Abbreviation	Definition
µg	microgram
µL	Microliter
µm	Micrometer
MDL	method detection limit
mg	Milligram
mL	Milliliter
mm	Millimeter
mmol	Millimole
min	minute
mol	Mole
MS	mass spectrometry
MS/MS	tandem mass spectrometry/mass spectrometry
ms	Millisecond
mV	Millivolt
MW	molecular weight
<i>m/z</i>	mass to charge ratio
n/a	not applicable
ND or nd	not detectable (below limit of detection)
ng	Nanogram
No.	Number
oz	Ounce
PMRA	Pest Management Regulatory Agency, Canada
ppb	parts per billion or micrograms per kilogram
ppm	parts per million or microgram per gram or milligrams per kilogram
pg	Picogram
psi	pounds per square inch
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

Abbreviations and symbols (continued)

Abbreviation	Definition
USDA	United States Department of Agriculture
UV	Ultraviolet
Vol	Volume
Wt	Weight
V	Volt

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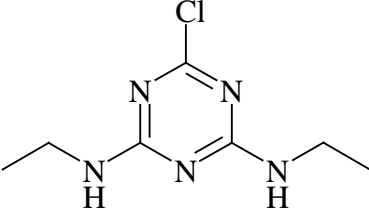
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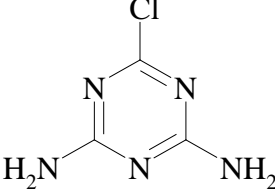
1.0 INTRODUCTION

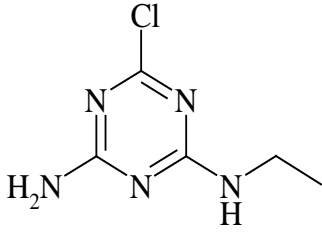
1.1 Scope and chemical structures

Analytical Method GRM052.01A (an updated method of AG-539, Reference 3) is suitable for the determination of residues of simazine, G28273 and G28279 in crops. The analyses of almonds, oranges, peaches, corn forage, corn grain, blueberries and avocados have been performed using this method. The limit of quantitation of the method has been established at 0.01 ppm. This method satisfies US EPA guideline OPPTS 860.1340 and EU Guidelines SANCO/3029/99 rev 4 and SANCO/825/00 rev 7.

The chemical structures of simazine, G28273 and G28279 and relevant information are summarized as follows:

Compound Structure	
Common Name	Simazine
CAS Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-
CAS Number:	122-34-9
Molecular Weight:	201.7
Standard Reference:	486779
Storage Conditions:	Frozen
Purity:	98.4%

Compound Structure	
Common Name	G28273
CAS Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-
CAS Number:	3397-62-4
Molecular Weight:	145.6
Standard Reference:	S-87-1195
Storage Conditions:	Frozen
Purity:	97.0%

Compound Structure	
Common Name	G28279
CAS Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-
CAS Number:	1007-28-9
Molecular Weight:	173.6
Standard Reference:	452369
Storage Conditions:	Frozen
Purity:	94.0%

1.2 Method summary

A 5 g sample is refluxed in methanol/water 80/20 (v/v) for two hours. Extracts are filtered and diluted with water/methanol + 0.1% formic acid 90:10 (v/v). Final determination is by high performance liquid chromatography with tandem mass spectrometry detection.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

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

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. 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.

2.3 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.

Prepare individual 100 µg/mL stock solutions for simazine, G28273 and G28279 by weighing 10 mg of analyte (corrected for purity) into a weighing dish and quantitatively transfer (using methanol) to a “Class A” volumetric flask (100 mL). Add additional methanol to the mark on the flask.

Prepare a combined 10 µg/mL fortification standard by adding 10 mL of each stock solution prepared as instructed above to a 100 mL volumetric flask and diluting to the mark with methanol. Prepare a combined 1 µg/mL fortification standard by adding 1 mL of each stock solution to a 100 mL volumetric flask and diluting to the mark with methanol. Prepare a combined 0.1 µg/mL fortification standard solution by adding 10 mL of the combined 1.0 µg/mL fortification standard to a 100 mL volumetric flask and diluting to the mark with methanol. The preparation of LC-MS/MS calibration standards is discussed in Section 3.7.

When not in use, always store standard solutions under refrigerated conditions to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use. Stock solutions expire 6 months after preparation.

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 MSDS or a monograph such as ‘Hazards in the Chemical Laboratory’, edited by S G Luxon, The Chemical Society, London (Reference 1).

Reagent/Solvent Hazards

Reagent/Solvent	Formic Acid	MeOH
Harmful Vapor	✓	✓
Highly Flammable	X	✓
Harmful by Skin Absorption	✓	✓
Syngenta Toxicity Class	C,S	B,S
OES Short Term (mg m ⁻³)	N/A	310
OES Long Term (mg m ⁻³)	9	260

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

3.0 ANALYTICAL PROCEDURE

3.1 Modifications and potential problems

To avoid possible suppression from matrix, samples should be diluted as much as possible. Increased dilution ratios can be achieved by individually diluting simazine and G28279 due to greater sensitivity. No significant suppression was encountered from the samples listed in this method with a 5x dilution from extract.

3.2 Sample preparation

Samples were prepared by randomly collecting a representative subsample. The samples were homogenized in a Hobart foodcutter using dry ice as necessary to keep the sample frozen. Samples were stored frozen (-20°C) prior to extraction.

See details in current revision of Syngenta SOP 7.21 “Preparation of Crop Samples for Residue Analysis”.

3.3 Extraction

- a) Weigh out a 5 gram representative subsample into a 500 mL round bottom flask. At least one untreated control and two control samples fortified by pipette with a known amount of simazine, G28273 and G28279 should be analyzed with each sample set to verify method performance. Add boiling chips. Typical fortification levels would include one at the method LOQ and one at 10X the LOQ.
- b) Add methanol/water 80:20 (v/v) (150 mL minus the water content of the sample) and place on a reflux apparatus for 120 minutes (2 hours). Alternatively, add 150 mL methanol and experimentally determine the moisture content of the sample to be calculated into the final extraction volume.
- c) Cool and filter samples through a Reeve Angel 802 inside a Whatman 2V filter into an 8 oz amber bottle. Refrigerate the supernatant if Section 3.4 procedures cannot be performed immediately.

3.4 Sample Dilution

- a) Transfer a 1.0 mL aliquot of crop extract and add 4.0 mL of water/methanol + 0.1% formic acid 90:10 (v/v) to make a final volume of 5 mL, vortex thoroughly. Additional dilutions may be performed depending on residue levels and instrument sensitivity.
- b) Transfer an aliquot from the final fraction into an injection vial for LC-MS/MS analysis. See Section 4.0 for instrument and operating conditions for analysis of simazine, G28273 and G28279.

3.5 Time required for analysis

The methodology is normally performed with a set of 12 samples. One person can complete the analysis of 1-2 sets in 8 working hours.

3.6 Method stopping points

The analytical procedure may be stopped after refluxing for overnight or weekend breaks. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored in a refrigerator in sealed containers when the analysis cannot be completed in a single day.

3.7 Preparation of calibration standards for LC-MS/MS

See preparation details for stock solutions and fortification standards in Section 2.3. A range of calibration standards should be prepared to develop a calibration curve for calculation of sample residues. Standards for external calibration should be prepared in water/methanol + 0.1% formic acid 90:10 (v/v). When not in use, these standard solutions should be stored refrigerated. The current instrumentation contains calibration standards that range from 0.04 pg/ μ L to 1.0 pg/ μ L.

Calibration standards should be stored in a refrigerator. An expiration date of 3 months is recommended.

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.

4.1 Instrument description

HPLC System	: Shimadzu UFLC XR
Detector	: Applied Biosystems API 5500 QTRAP with Analyst Software (version 1.5)

4.2 Chromatography conditions

<u>HPLC Pump:</u>	Shimadzu UFLC XR System
<u>Mobile Phase A:</u>	0.1% Formic Acid in H ₂ O
<u>Mobile Phase B:</u>	0.1% Formic Acid in MeOH
<u>Flow Rate:</u>	1.0 mL/min
<u>Column:</u>	Zorbax SB-AQ 4.6 x 50mm (3.5 μ)
<u>Column Oven Temp:</u>	25°C
<u>Injection Vol.</u>	10 μ L
<u>Run Time:</u>	5 minutes
<u>Retention Time:</u>	G28273 1.8 minutes
	G28279 2.3 minutes
	Simazine 2.7 minutes

Mobile Phase Composition

A gradient elution using a linear curve with increasing percentage of organic solvent will be used. See the specific gradient listed below:

Time	%A	%B
0.0	95	5
2.5	5	95
3.3	5	95
3.7	95	5
5.0	95	5

Note: Retention times may differ depending upon the flow rate, column, and gradient used.

4.3 Mass spectrometer conditions

Detection: TurboSpray Ionization positive (+) mode Multiple Reaction Monitoring (MRM) as follows:

Analyte	Q1 MS (Molecular)	Q3 MS (Product)	Dwell Time	DP	CE
Simazine	m/z = 202.1	m/z = 132.0	25 ms	50	25
G28273	m/z = 146.0	m/z = 104.0	50 ms	71	27
G28279	m/z = 174.1	m/z = 104.0	25 ms	50	30

MS/MS Conditions: Source Temperature: 600 °C
CAD Gas: MED
Curtain Gas: 20
GS1: 70
GS2: 50
IS: 1000
Q1 Resolution: Unit
Q3 Resolution: Unit
Entrance Potential: 10
CXP: 10

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 1.5) to the Analysis Worksheet or LIMS System to calculate the final results.

5.0 CALCULATION OF RESULTS

This method was developed using a multipoint calibrating procedure. Residue weight concentrations may be calculated in ppb or ppm for each sample as follows.

- a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples. The recommended concentration range is located in Section 3.7.
- 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 six injections of sample solutions.
- c) Generate calibration curve parameters using an appropriate regression package.
Note: A square linear regression is strongly recommended.
- d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

- e) Where y is the instrument response value, x is the Sample Concentration, m is the slope of the line of best fit (“X-variable 1” in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the “R-Squared” value for the regression.
- f) Re-arrangement for x gives

$$\text{Sample Conc} = x = \frac{y - c}{m}$$

- g) To calculate the residue results, the sample concentration must first be calculated as follows:

$$\text{Sample Conc}(g / mL) = \left(\frac{\text{Sample wt}(g)}{\text{extraction volume}^*(mL)} \right) \times \left(\frac{\text{aliquot volume}(mL)}{\text{final volume}(mL)} \right)$$

* *Extraction volume = extraction volume + sample weight x moisture*

To determine the residue of each analyte in the samples, use the following equation:

$$\text{Residue}(ppb) = \frac{\text{Calculated Conc}(ng / mL)}{\text{Sample Conc}(g/mL)}$$

6.0 UNTREATED CONTROL AND RECOVERY EXPERIMENTS

Untreated control samples, if available, should be analyzed for each set of samples analyzed to verify that samples are free from analyte contamination. A minimum of one control should be analyzed with each set of samples.

At least two recovery samples (untreated samples accurately fortified by pipette with a known amount of analytes prior to extraction) should also be analyzed alongside each set of samples, one fortified at the method LOQ and one fortified at the expected level of residues.

To determine the recovery factor, first determine the concentration in the aliquot of the sample injected, and calculate the sample concentration injected using the procedures described in Section 3.0. Calculate the final residue values found in the control and 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 factor as a percentage (R%) by the equation:

$$\text{Recovery} = \frac{\text{Analyte (ppb) found in Sample} - \text{Analyte (ppb) found in Untreated Control}}{\text{Analyte (ppb) Spiked}}$$

Recovery efficiency is acceptable when the mean values are between 70% and 120% and with a relative standard deviation of $\leq 20\%$, based on EPA Guidelines OPPTS 860.1340.

7.0 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 run on a routine basis.

7.1 Matrix

Interference arising from the crop matrices tested has not been observed. If suppression due to matrix is encountered greater than 10%, further dilution or a cleanup step will be needed.

7.2 Reagent and solvent interference

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

7.3 Labware interference

This method uses disposable labware, where possible. All reusable glassware should be detergent washed and then rinsed with in-house deionized (HYDRO™ purified water) and methanol prior to use.

8.0 METHOD VALIDATION

A method validation study (TK0027483) has been carried out on the procedures described in this method for analysis of simazine, G28273 and G28279 in crops including almonds, oranges, peaches, corn forage, corn grain, blueberries and avocados. The following statistics are based on simazine, G28273 and G28279 validation data from TK0027483 (Reference 2).

8.1 Accuracy and Precision

The accuracy of the method is determined by procedural recovery values. The table below contains the range of recoveries at 0.01 ppm and 0.10 ppm determined in the validation study (Reference 2):

Analyte	Mean Recovery Range
Simazine	86-106%
G28273	90-104%
G28279	90-100%

These results show that the procedures in this method can be used to accurately determine actual residues in the crops analyzed.

The precision of the method was determined by evaluating the average recovery, standard deviation and the relative standard deviation (RSD) in the validation studies (Reference 2) as detailed below:

Analyte	Mean Recovery (n = 70)	STD	RSD (%)
Simazine	98%	8.6	8.7
G28273	96%	8.7	9.0
G28279	96%	7.2	7.6

These results show that residues can be determined with good reproducibility.

8.2 Limit of quantitation (LOQ)

The LOQ of the method 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 has been set at 0.01 ppm for analysis of simazine, G28273 and G28279.

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, compound to compound, and

from instrument to instrument. A LOD of 0.4 pg was established using the instrumentation contained in this method. Lower LOD's for simazine and G28279 may be determined based on better sensitivity over G28273. Injections of 0.40 pg standard can be reliably quantitated with a signal to noise ratio of at least 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 set injected. It has been shown that the LC-MS/MS detector responses are generally linear in the range from 0.40 pg to 10.0 pg injected on column for simazine, G28273 and G28279.

9.0 LIMITATIONS

The method has been tested on representative crops and fruits. It can reasonably be assumed that the method can be applied for other matrices not tested in this method provided that successful recovery tests are performed at the relevant levels to 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 simazine, G28273 and G28279 in almonds (nutmeat), oranges (fruit), peaches (fruit), corn (forage), corn (grain), blueberries (fruit), and avocados (fruit). Only commercially available laboratory equipment and reagents are required. The analyses of 1 to 2 sets can be completed by one person in a day (8 working hour period). Untreated and fortified samples should be analyzed with each set to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantitation (LOQ) of the method has been established at 0.01 ppm for analysis of simazine, G28273 and G28279.

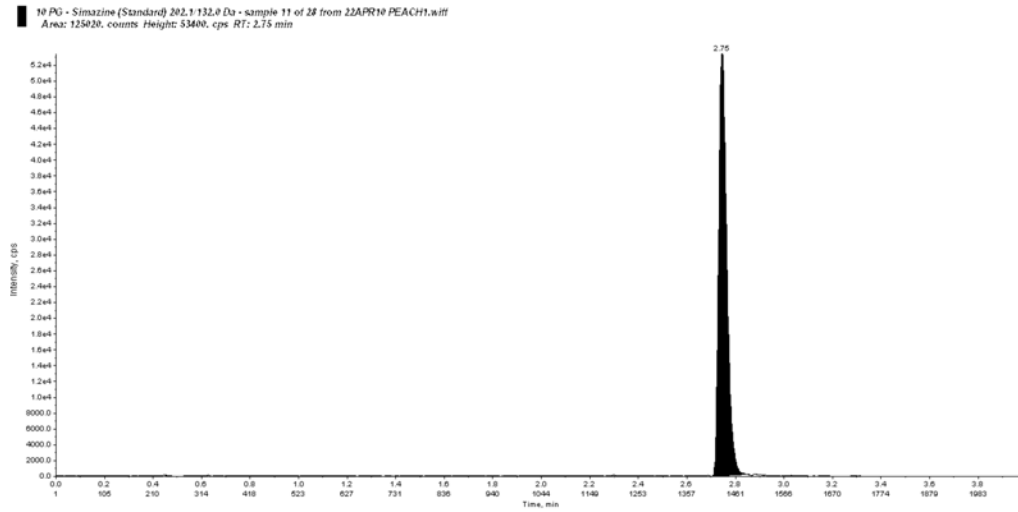
This method satisfies US EPA guideline OPPTS 860.1340 and EU Guidelines SANCO/3029/99 rev 4 and SANCO/825/00 rev 7.

11.0 REFERENCES

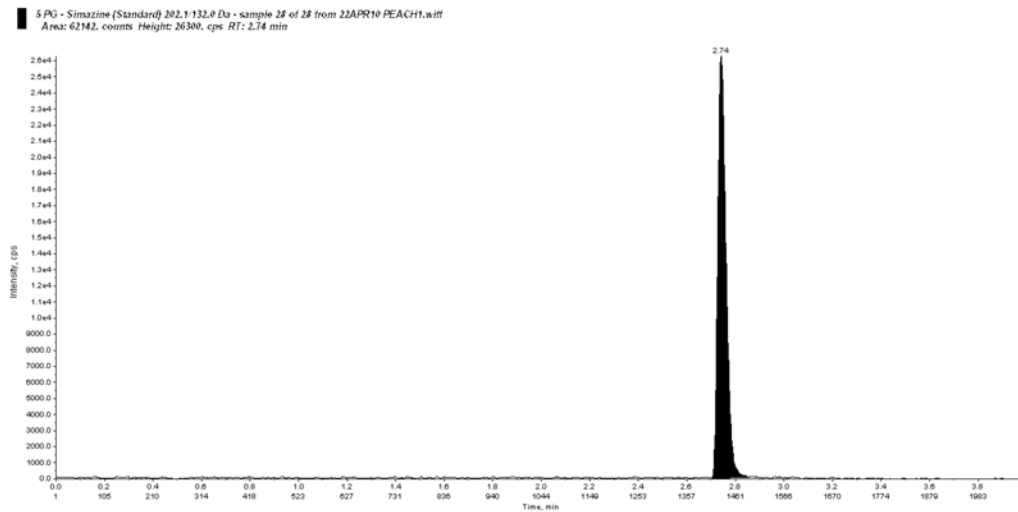
1. Luxon, S G (1992): Hazards in the Chemical Laboratory 5th Edition. The Royal Society of Chemistry. Thomas Graham House, The Science Park, Cambridge CB4 4WF, UK. ISBN 0-85186-229-2.
2. Mayer, L (2010), Syngenta Final Report, "Validation Of Analytical Method GRM052.01A for the Determination of Simazine, G28273 and G29279 in Crops. Final Determination by LC-MS/MS"
3. Lam KC, and Gold B (1988): Method AG-539, Determination of Simazine, G28279 and G28273 Residues In Vegetables, Fruit, Grains, and Crop Fractions Using Capillary Gas Chromatography. CIBA-GEIGY Corporation, Greensboro, NC.

FIGURES SECTION

FIGURE 1 **Representative Chromatograms – Simazine Calibration Standards. Transition $m/z = 202.1 \rightarrow 132.0$**

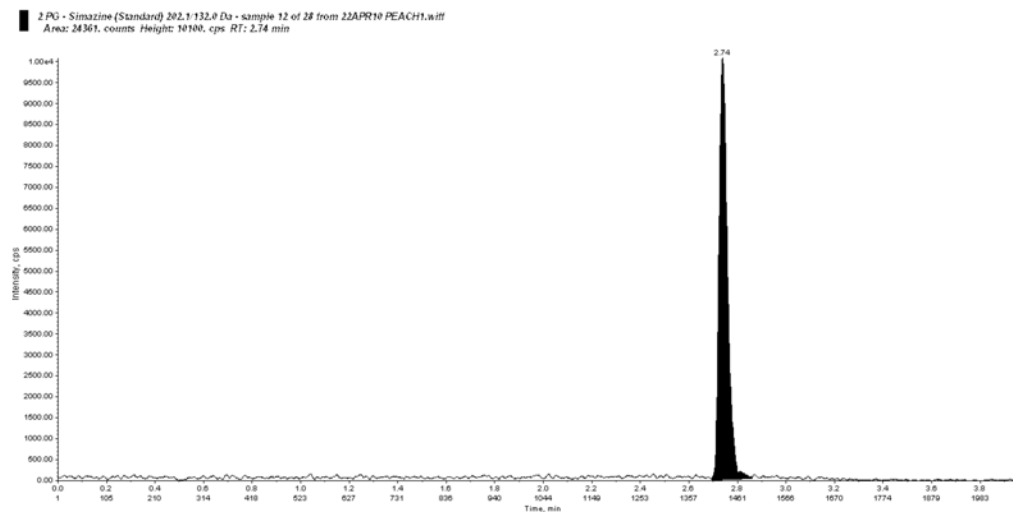


1. Standard (1.0 pg/ μ L), 10 μ L injection volume, 10.0 pg of simazine injected, peak area = 125020, from analysis of peach.

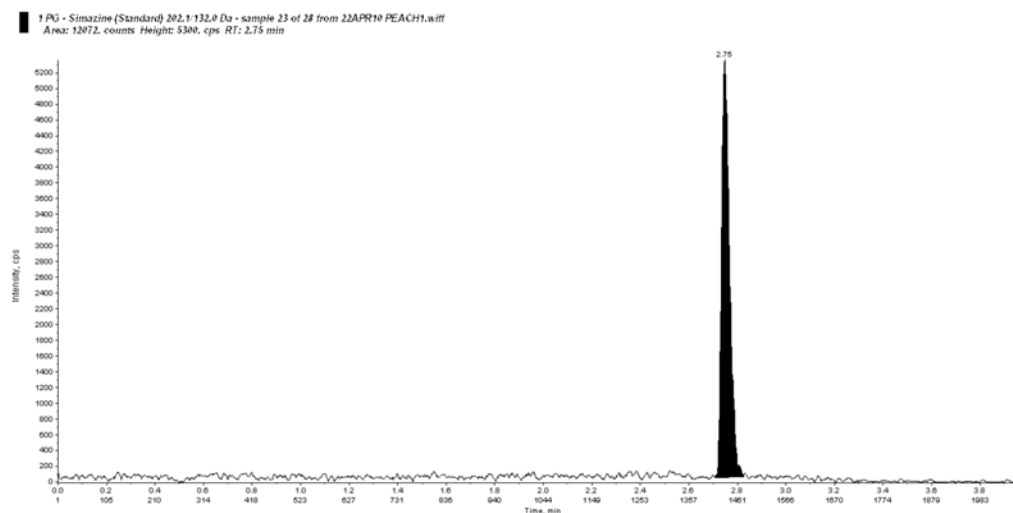


2. Standard (0.50 pg/ μ L), 10 μ L injection volume, 5.0 pg of simazine injected, peak area = 62142, from analysis of peach.

FIGURE 1 **Representative Chromatograms – Simazine Calibration Standards. Transition $m/z = 202.1 \rightarrow 132.0$ (Continued)**

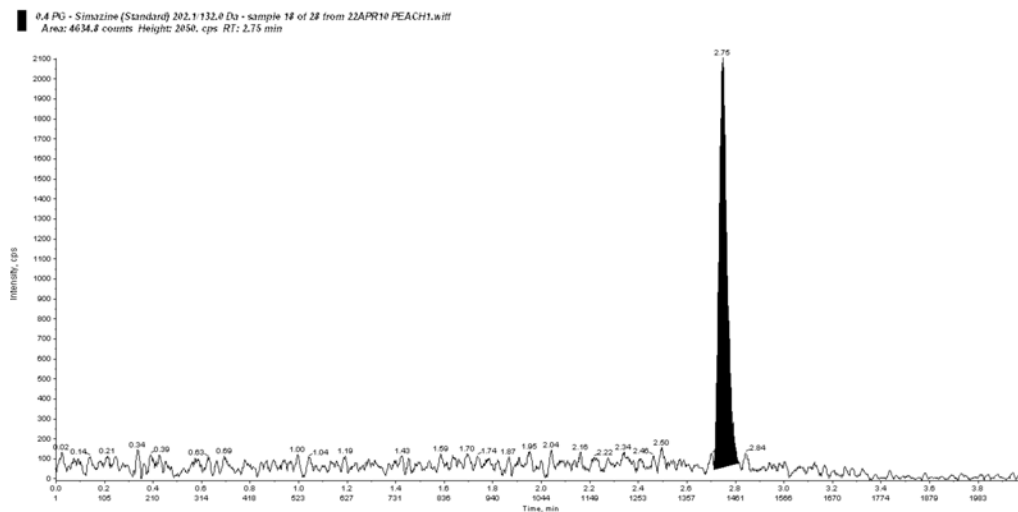


3. Standard (0.20 pg/ μ L), 10 μ L injection volume, 2.0 pg of simazine injected, peak area = 24361, from analysis of peach.



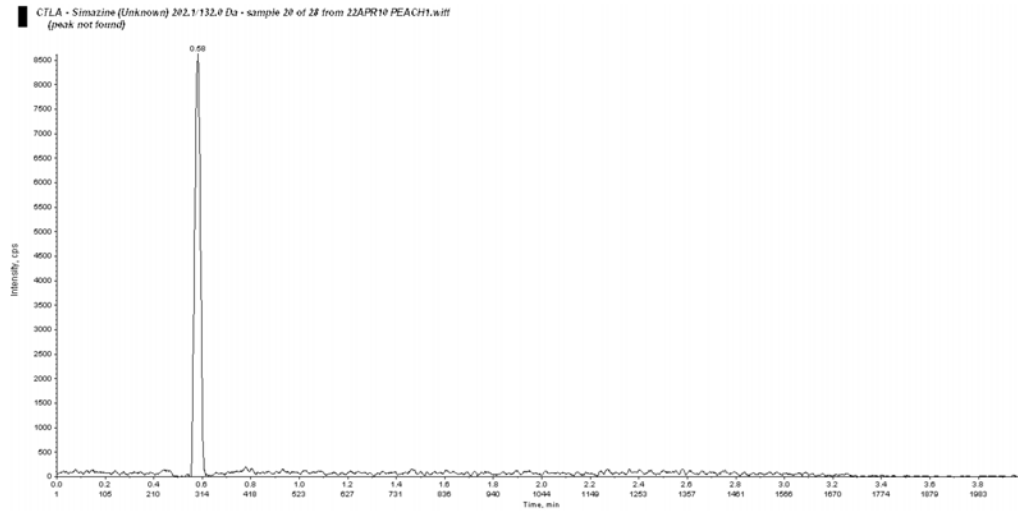
4. Standard (0.10 pg/ μ L), 10 μ L injection volume, 1.0 pg of simazine injected, peak area = 12072, from analysis of peach.

**FIGURE 1 Representative Chromatograms – Simazine Calibration Standards.
Transition $m/z = 202.1 \rightarrow 132.0$ (Continued)**

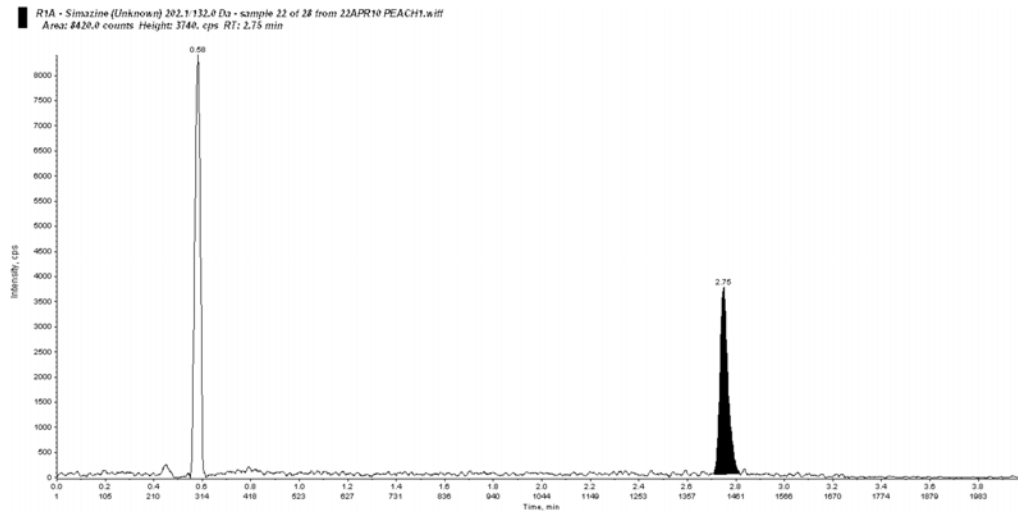


5. Standard (0.05 pg/ μ L), 8 μ L injection volume, 0.40 pg of simazine injected, peak area = 4635, from analysis of peach.

FIGURE 2 **Representative Chromatograms – Simazine Peach Transition**
 $m/z = 202.1 \rightarrow 132.0$

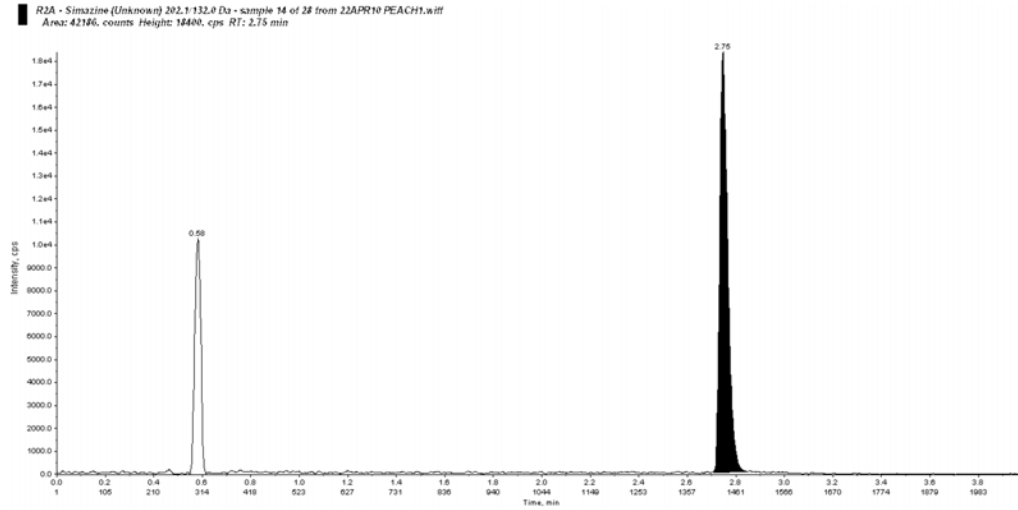


1. Peach Control, <0.01 ppm determined, Set 22APR10 PEACH1.

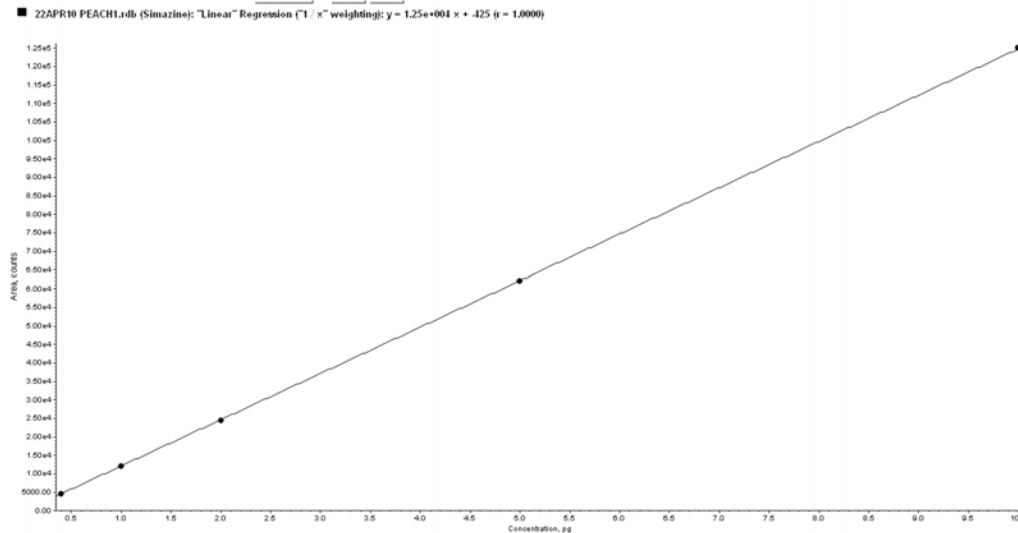


2. Peach Control + 0.01 ppm R1A, 109% Recovery, Set 22APR10 PEACH1.

FIGURE 2 **Representative Chromatograms – Simazine Peach Transition**
 $m/z = 202.1 \rightarrow 132.0$ (Continued)

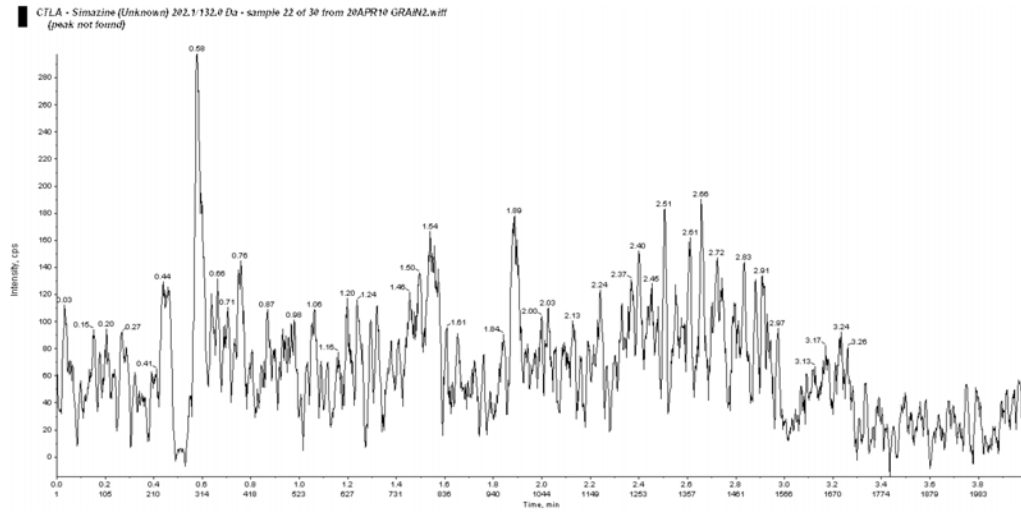


3. Peach Control + 0.10 ppm R2A, 105% Recovery, Set 22APR10 PEACH1.

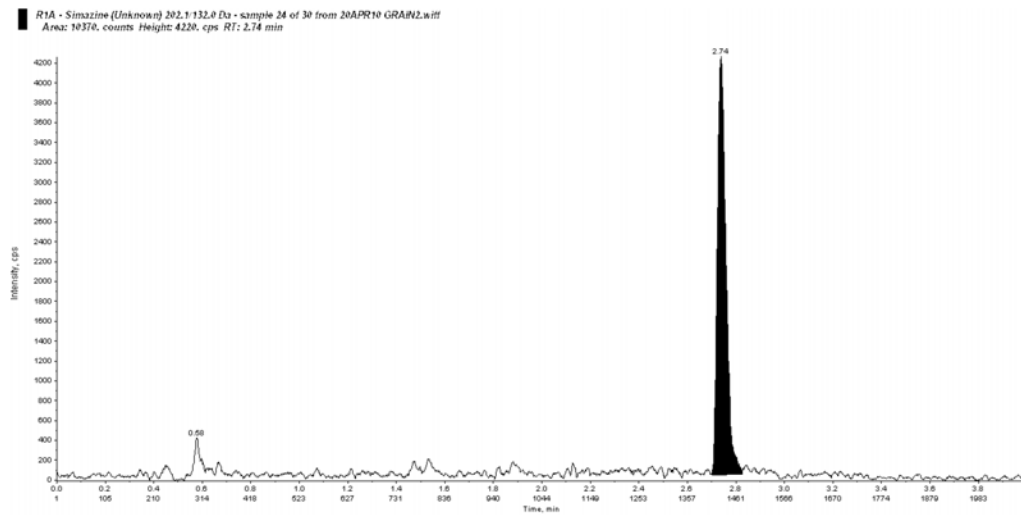


Simazine Peach Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of simazine standards.

FIGURE 3 **Representative Chromatograms – Simazine Corn Grain**
Transition $m/z = 202.1 \rightarrow 132.0$

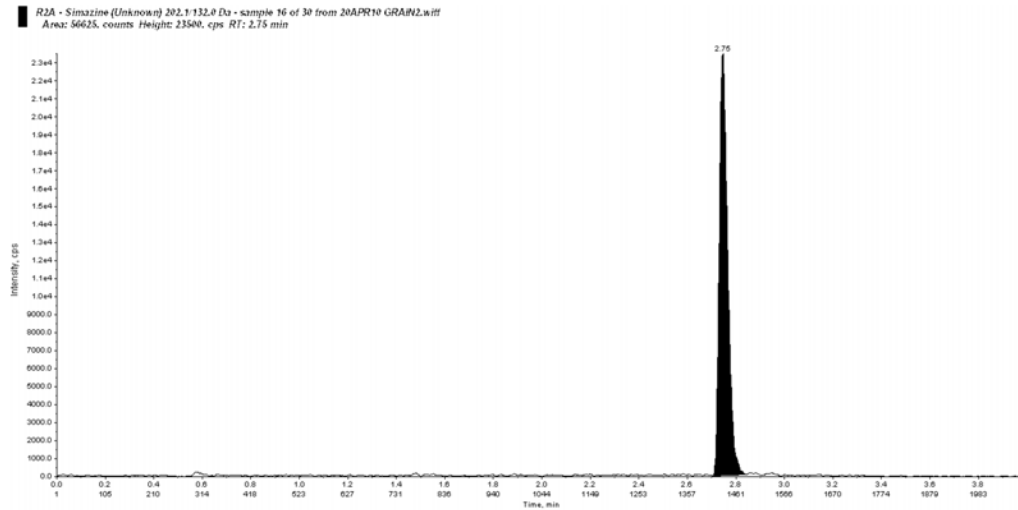


1. Corn Grain Control, <0.01 ppm determined, Set 20APR10 GRAIN2.

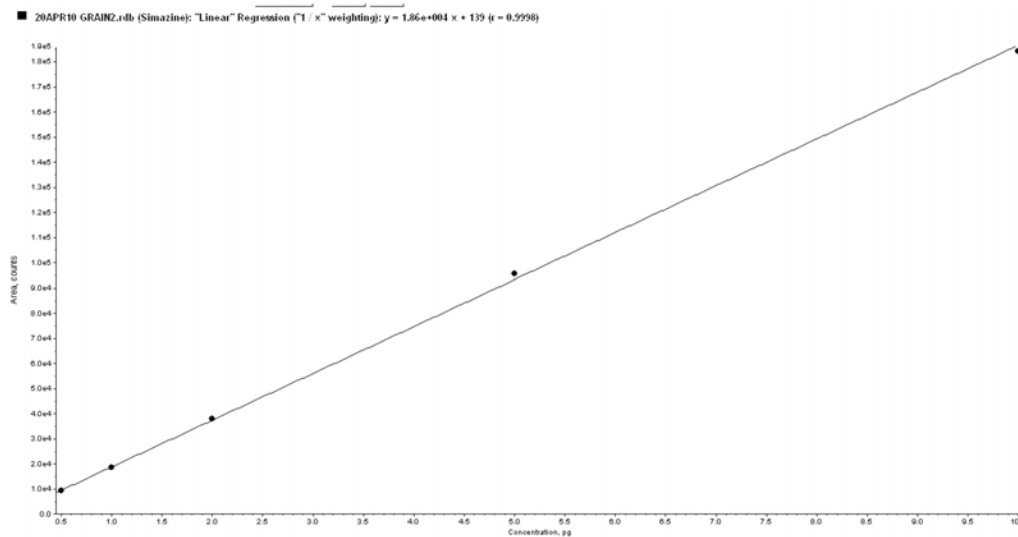


2. Corn Grain Control + 0.01 ppm R1A, 83% Recovery, Set 20APR10 GRAIN2.

FIGURE 3 **Representative Chromatograms – Simazine Corn Grain
Transition $m/z = 202.1 \rightarrow 132.0$ (Continued)**

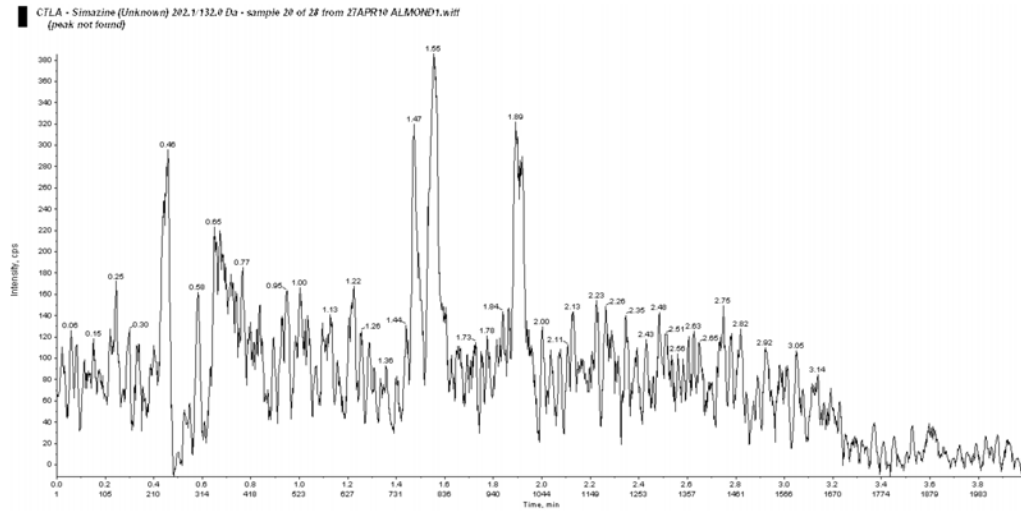


3. Corn Grain Control + 0.10 ppm R2A, 91% Recovery, Set 20APR10 GRAIN2.

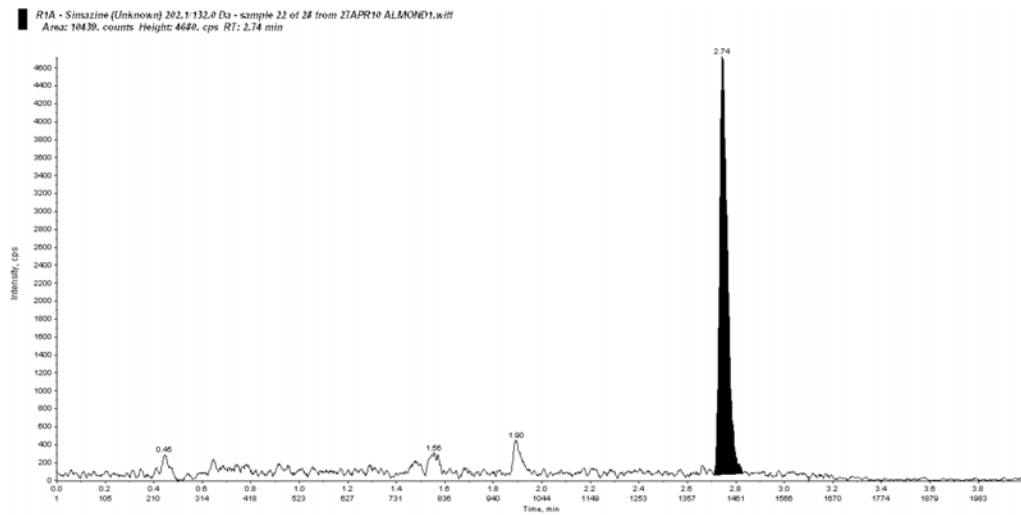


Simazine Corn Grain Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of simazine standards.

FIGURE 4 **Representative Chromatograms – Simazine Almond Transition**
 $m/z = 202.1 \rightarrow 132.0$

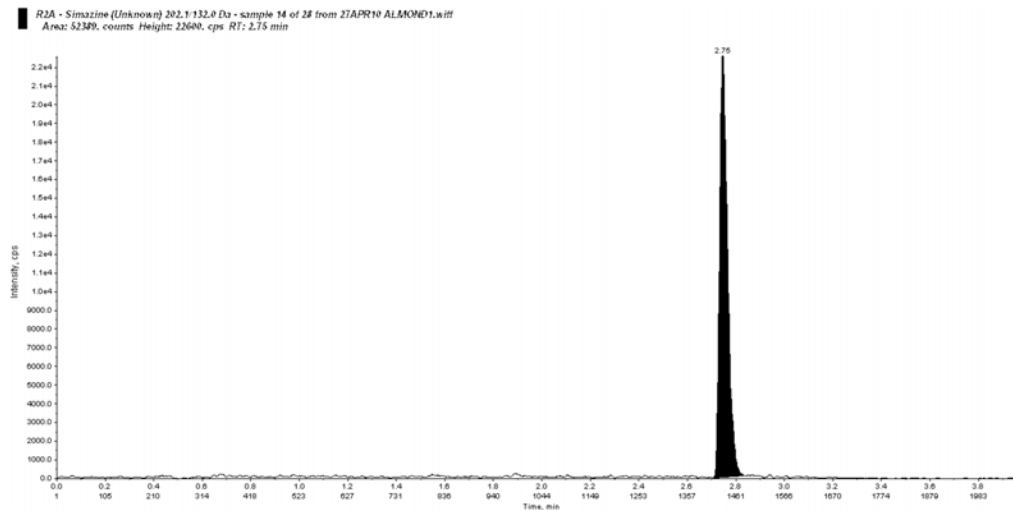


1. Almond Control, <0.01 ppm determined, Set 27APR10 ALMOND1.

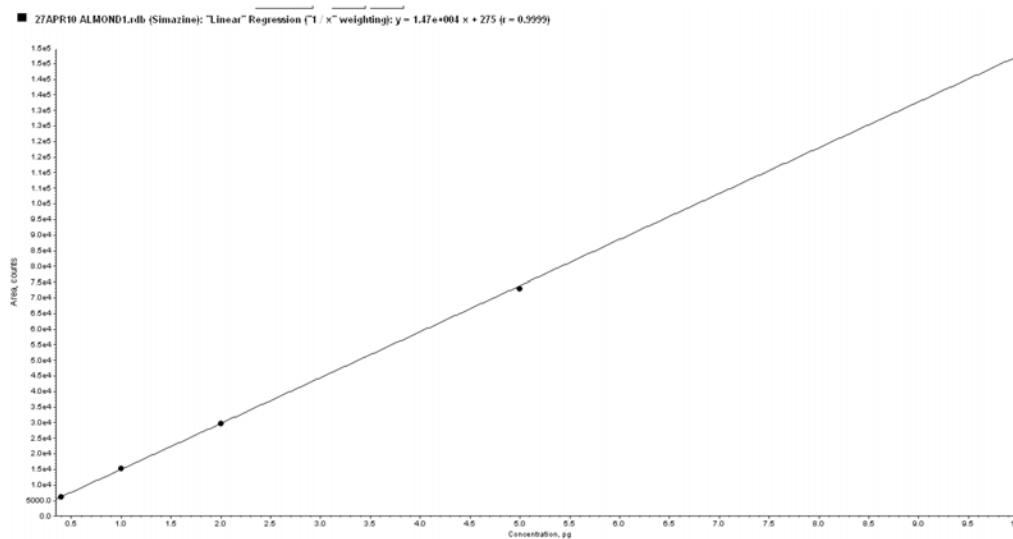


2. Almond Control + 0.01 ppm R1A, 104% Recovery, Set 27APR10 ALMOND1.

FIGURE 4 **Representative Chromatograms – Simazine Almond Transition**
 $m/z = 202.1 \rightarrow 132.0$ (Continued)

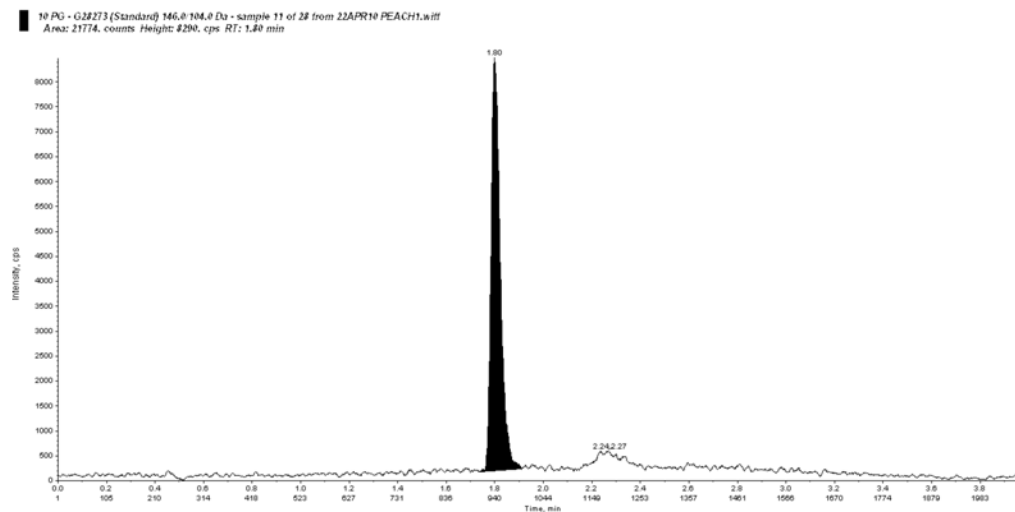


3. Almond Control + 0.10 ppm R2A, 106% Recovery, Set 27APR10 ALMOND1.

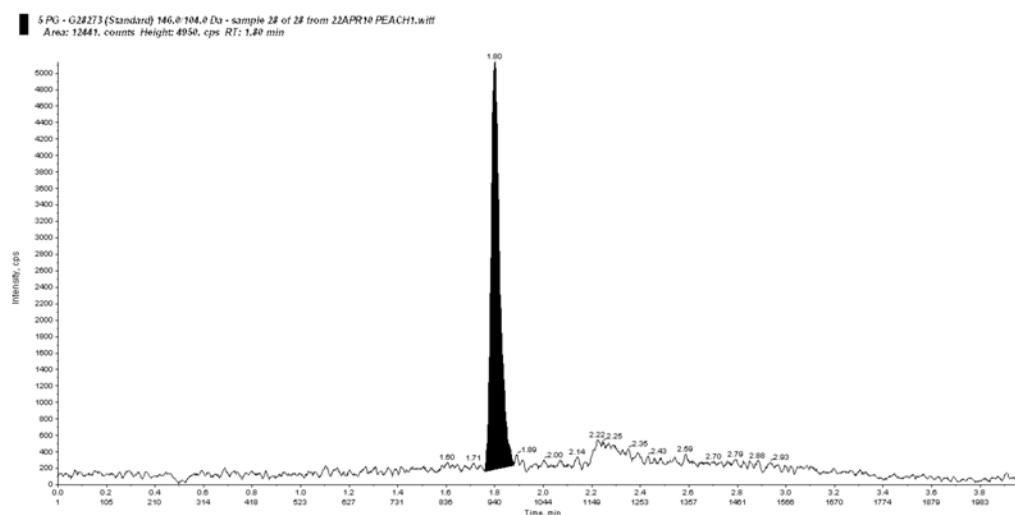


Simazine Almond Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of simazine standards.

FIGURE 5 **Representative Chromatograms – G28273 Calibration Standards. Transition $m/z = 146.0 \rightarrow 104.0$**

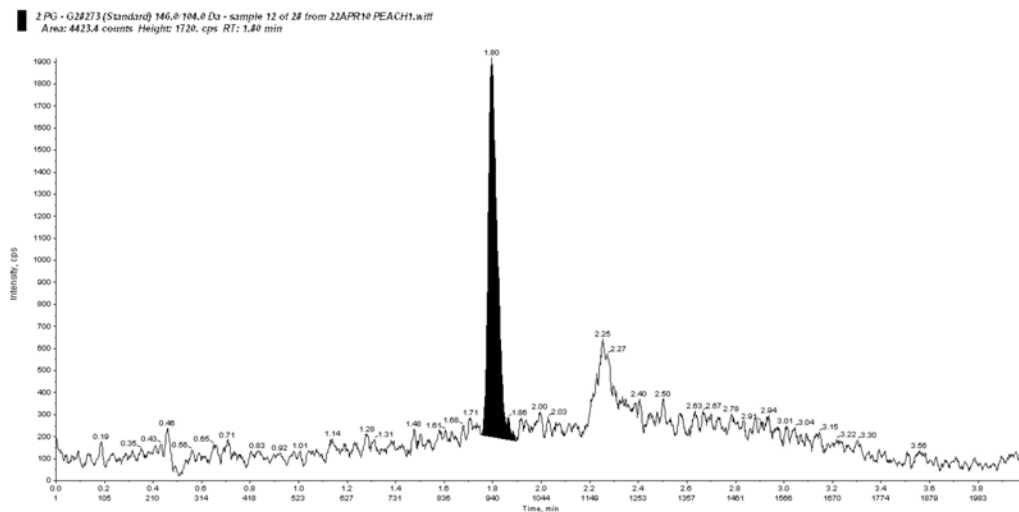


1. Standard (1.0 pg/ μ L), 10 μ L injection volume, 10.0 pg of G28273 injected, peak area = 21774, from analysis of peach.

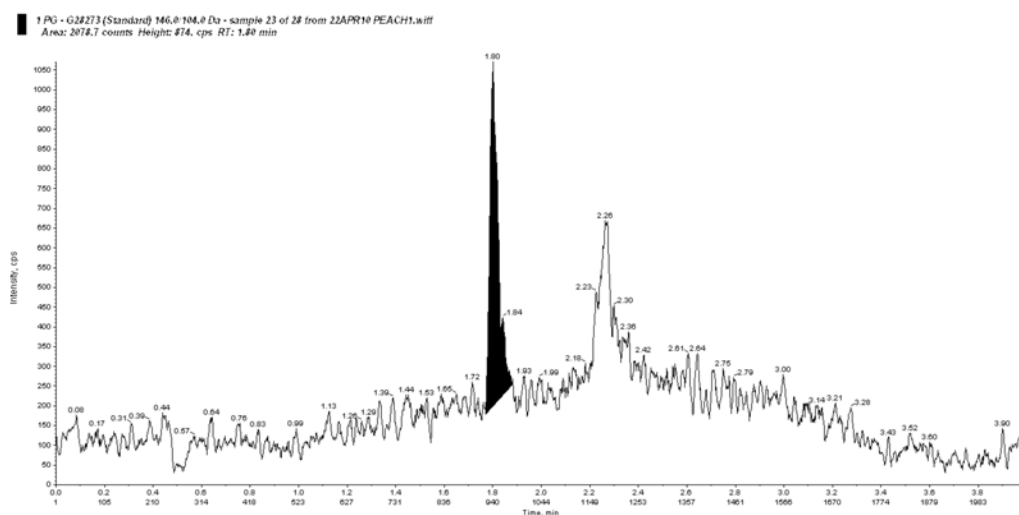


2. Standard (0.50 pg/ μ L), 10 μ L injection volume, 5.0 pg of G28273 injected, peak area = 12441, from analysis of peach.

**FIGURE 5 Representative Chromatograms – G28273 Calibration Standards.
Transition $m/z = 146.0 \rightarrow 104.0$ (Continued)**

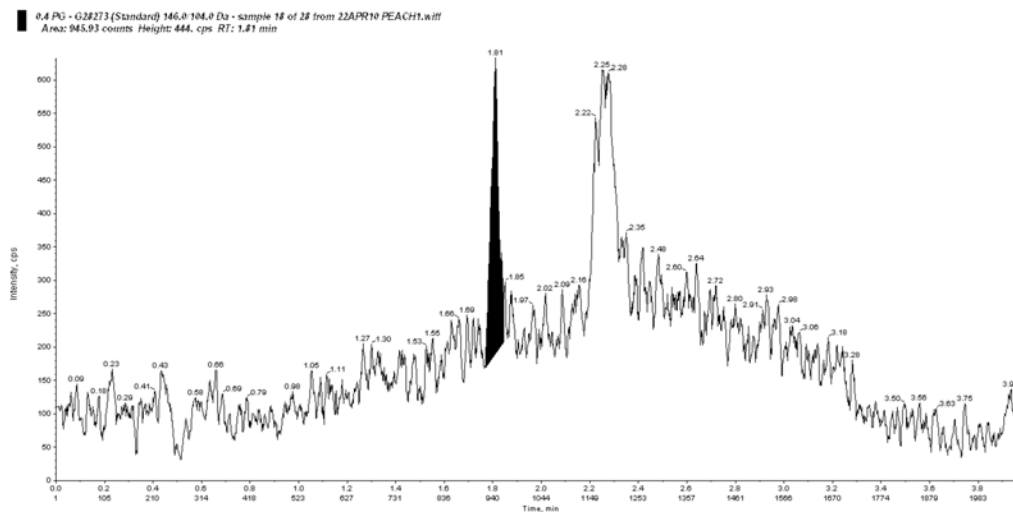


3. Standard (0.20 pg/ μ L), 10 μ L injection volume, 2.0 pg of G28273 injected, peak area = 4423, from analysis of peach.



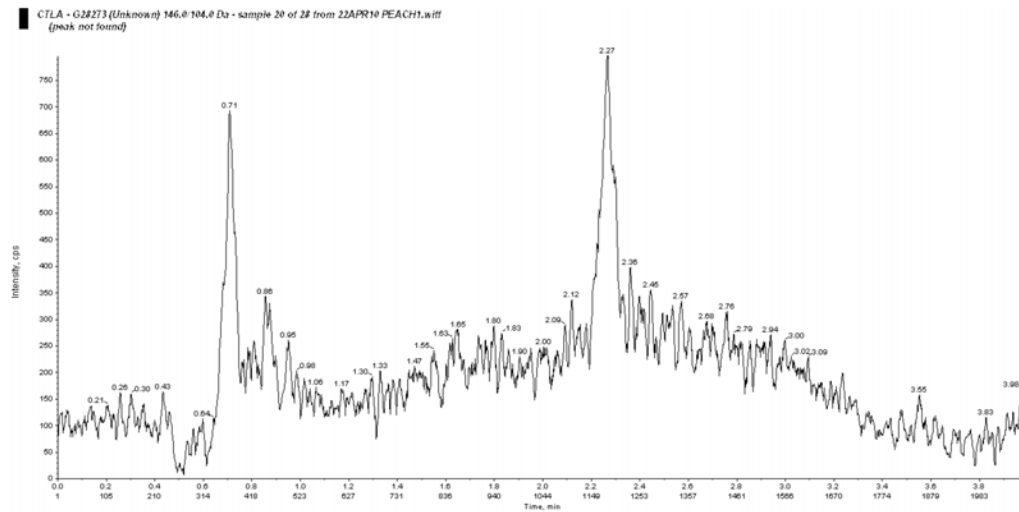
4. Standard (0.10 pg/ μ L), 10 μ L injection volume, 1.0 pg of G28273 injected, peak area = 2079, from analysis of peach.

**FIGURE 5 Representative Chromatograms – G28273 Calibration Standards.
Transition $m/z = 146.0 \rightarrow 104.0$ (Continued)**

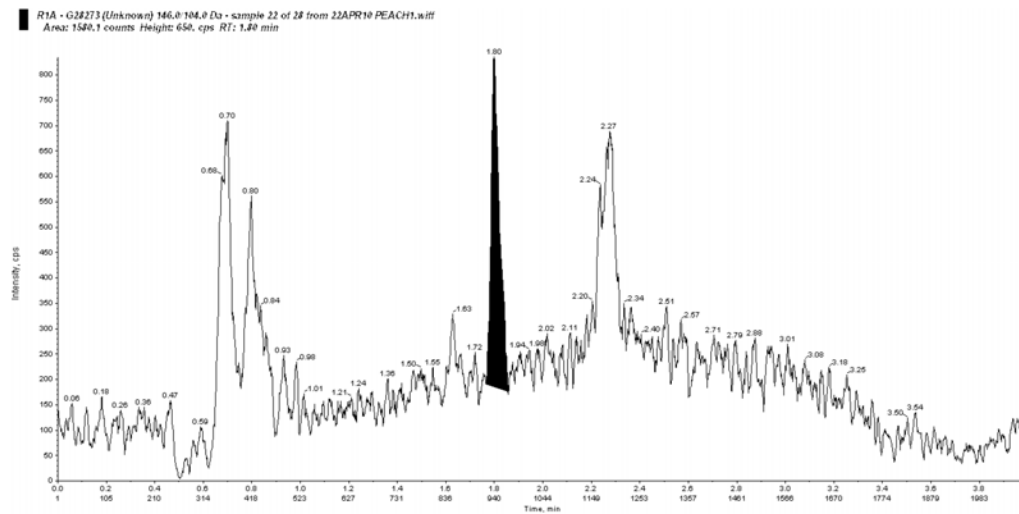


5. Standard (0.05 pg/ μ L), 8 μ L injection volume, 0.40 pg of G28273 injected, peak area = 946, from analysis of peach.

FIGURE 6 **Representative Chromatograms – G28273 Peach Transition m/z = 146.0→104.0**

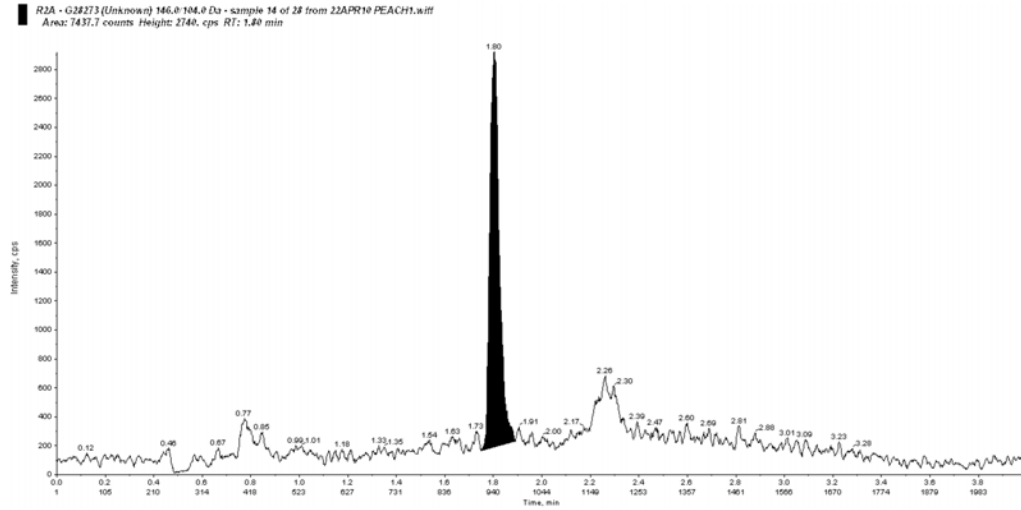


1. Peach Control, <0.01 ppm determined, Set 22APR10 PEACH1.

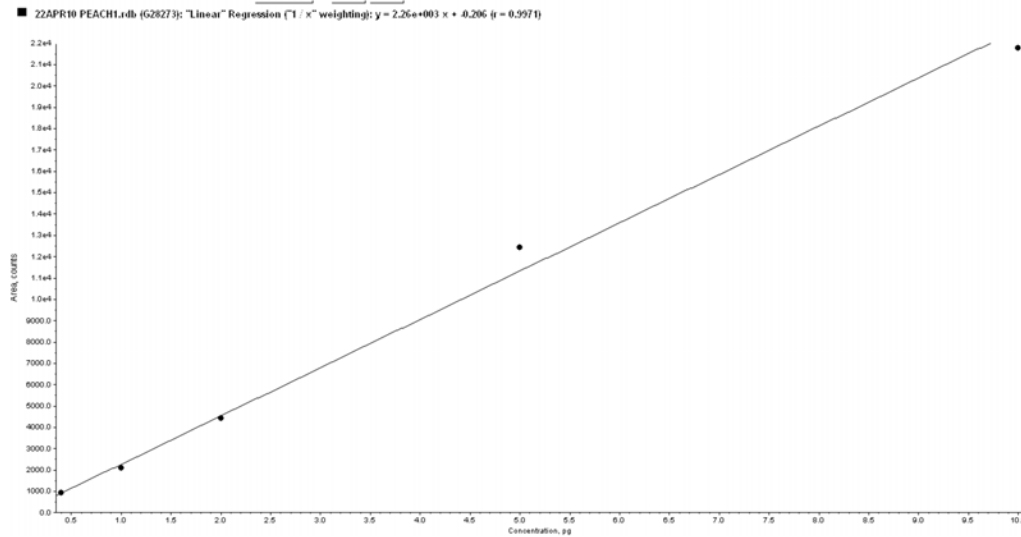


2. Peach Control + 0.01 ppm R1A, 99% Recovery, Set 22APR10 PEACH1.

FIGURE 6 **Representative Chromatograms – G28273 Peach Transition m/z = 146.0→104.0 (Continued)**

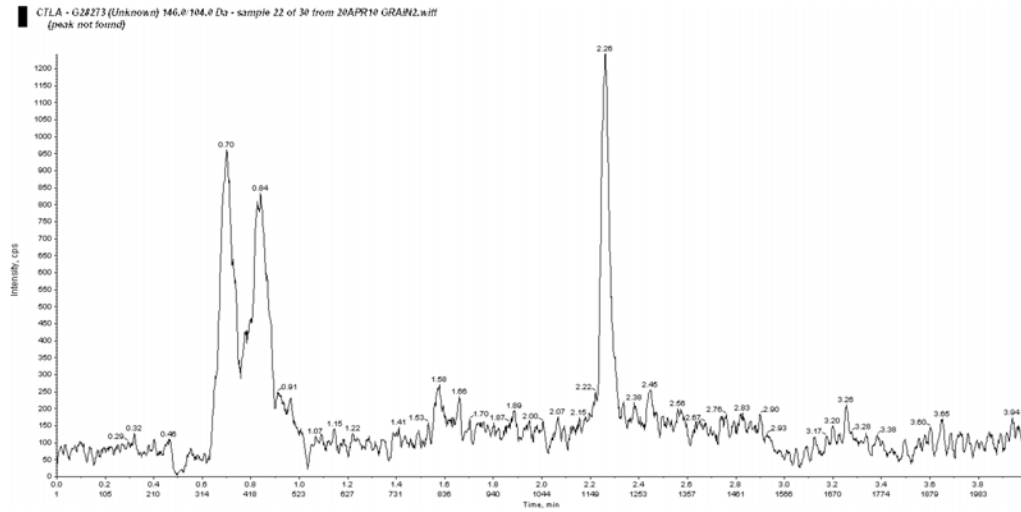


3. Peach Control + 0.10 ppm R2A, 101% Recovery, Set 22APR10 PEACH1.

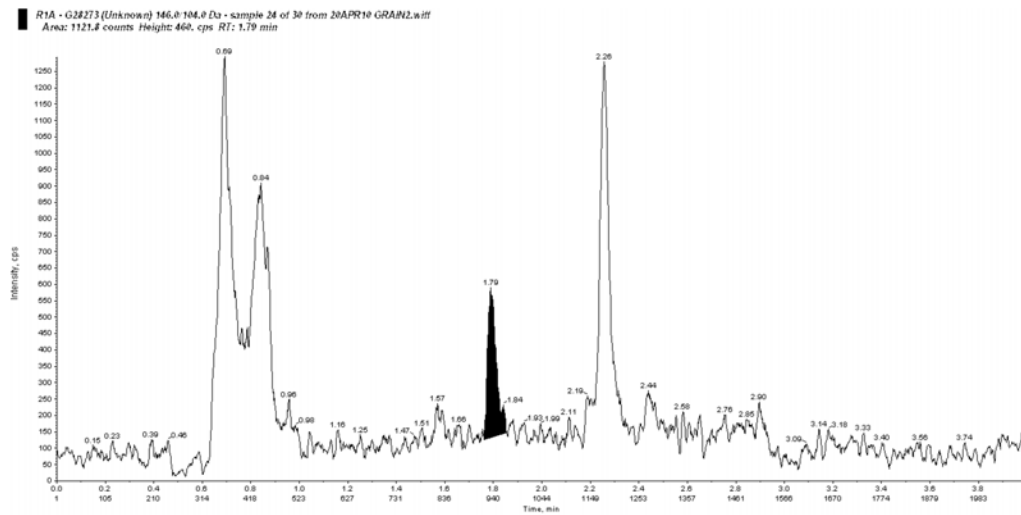


G28273 Peach Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of G28273 standards.

FIGURE 7 **Representative Chromatograms – G28273 Corn Grain**
Transition $m/z = 146.0 \rightarrow 104.0$

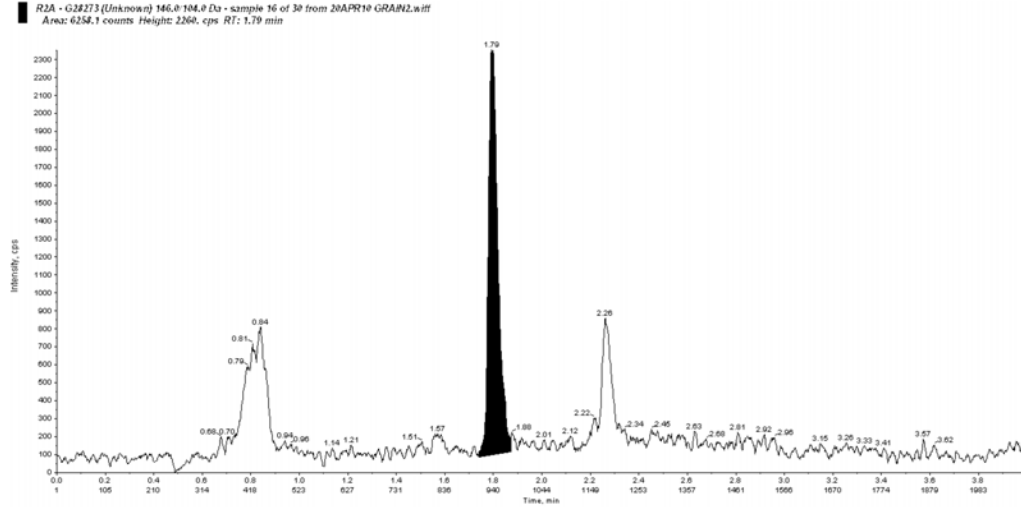


1. Corn Grain Control, <0.01 ppm determined, Set 20APR10 GRAIN2.

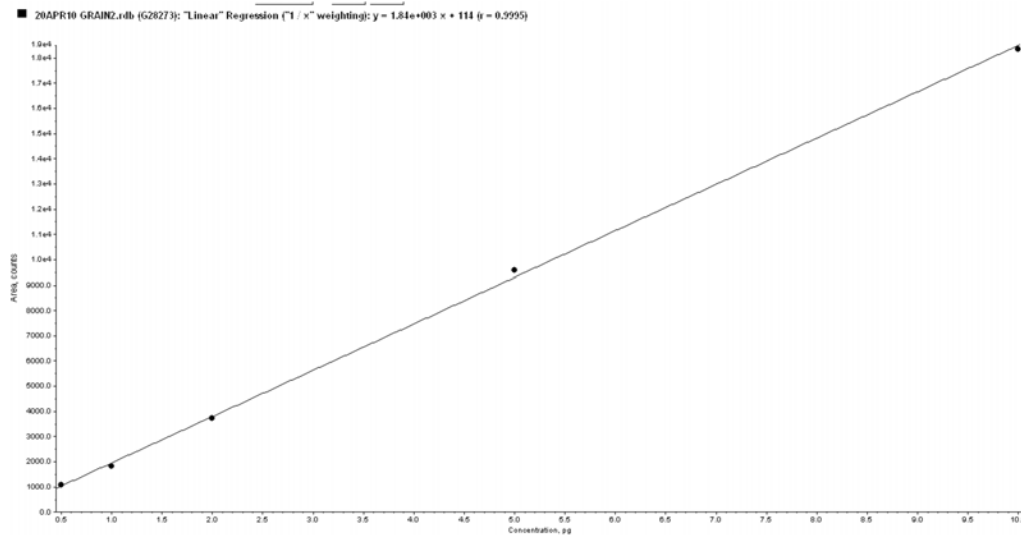


2. Corn Grain Control + 0.01 ppm R1A, 83% Recovery, Set 20APR10 GRAIN2.

FIGURE 7 **Representative Chromatograms – G28273 Corn Grain Transition $m/z = 146.0 \rightarrow 104.0$ (Continued)**

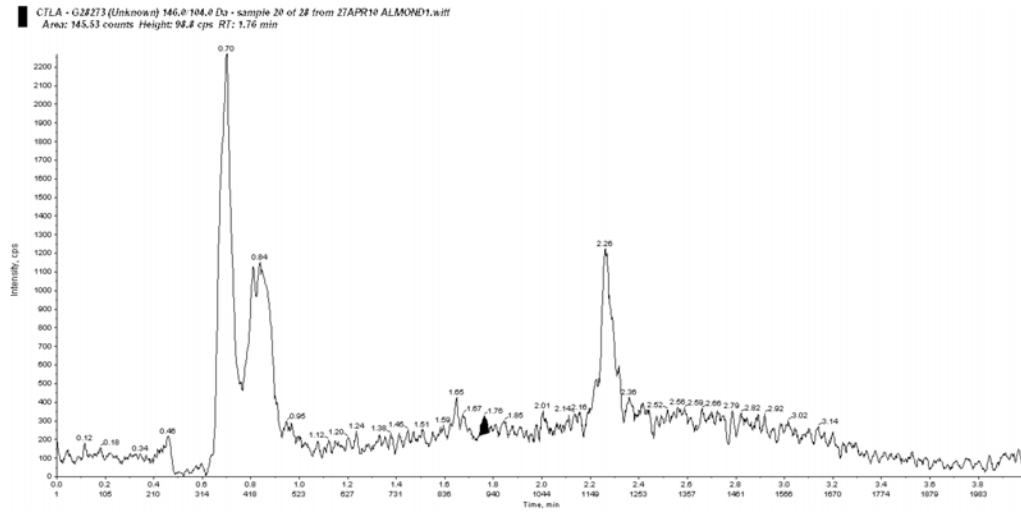


3. Corn Grain Control + 0.10 ppm R2A, 101% Recovery, Set 20APR10 GRAIN2.

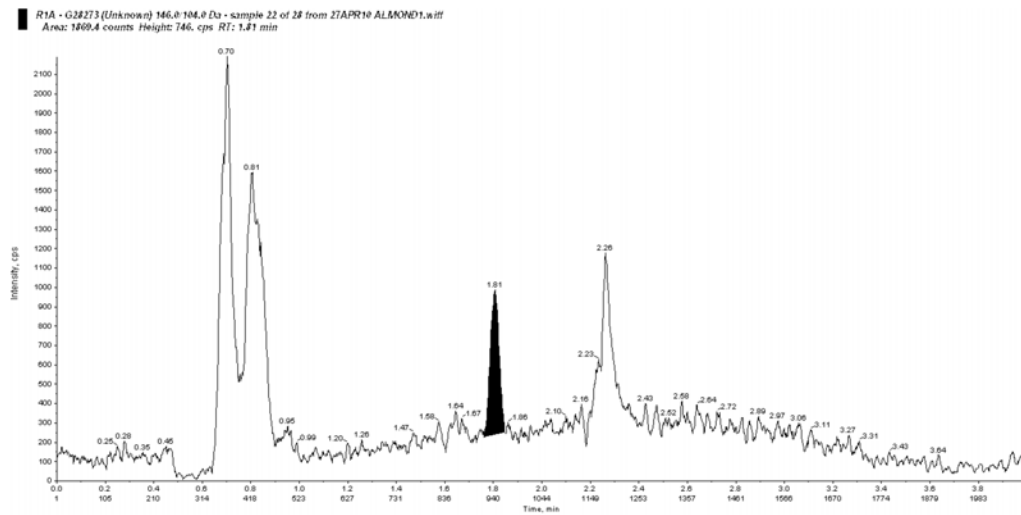


G28273 Corn Grain Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of G28273 standards.

FIGURE 8 **Representative Chromatograms – G28273 Almond Transition**
 $m/z = 146.0 \rightarrow 104.0$

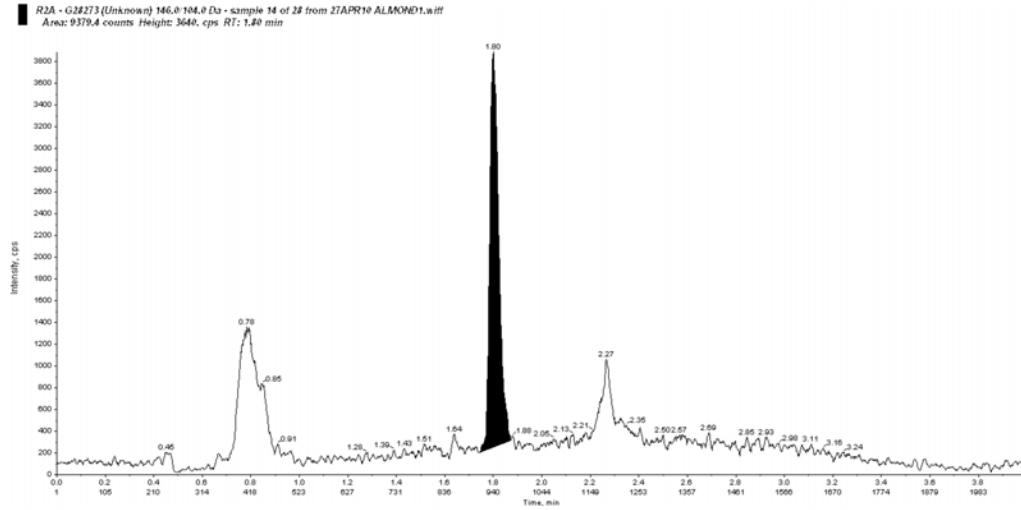


1. Almond Control, <0.01 ppm determined, Set 27APR10 ALMOND1.

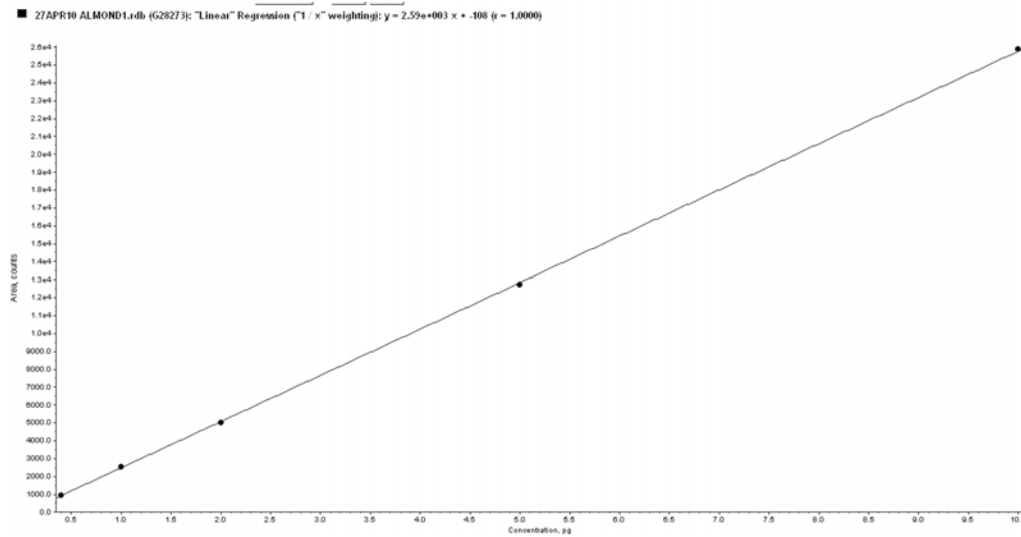


2. Almond Control + 0.01 ppm R1A, 100% Recovery, Set 27APR10 ALMOND1.

FIGURE 8 **Representative Chromatograms – G28273 Almond Transition**
 $m/z = 146.0 \rightarrow 104.0$ (Continued)

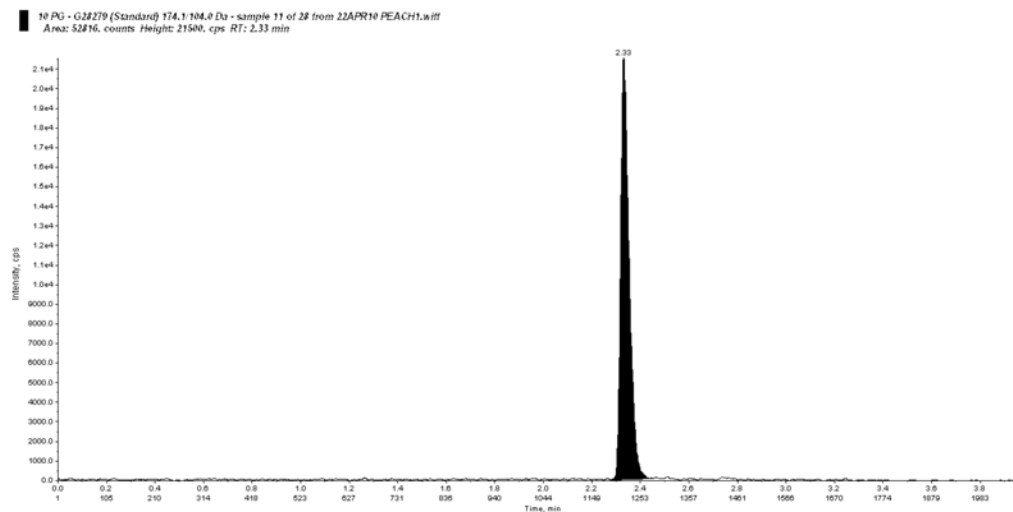


3. Almond Control + 0.10 ppm R2A, 109% Recovery, Set 27APR10 ALMOND1.

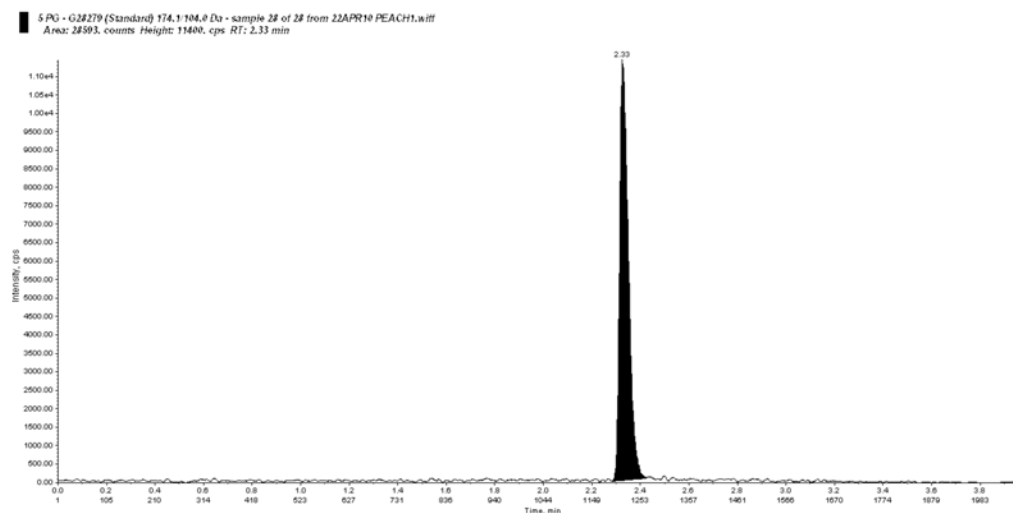


G28273 Almond Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of G28273 standards.

FIGURE 9 **Representative Chromatograms – G28279 Calibration Standards. Transition $m/z = 174.1 \rightarrow 104.0$**

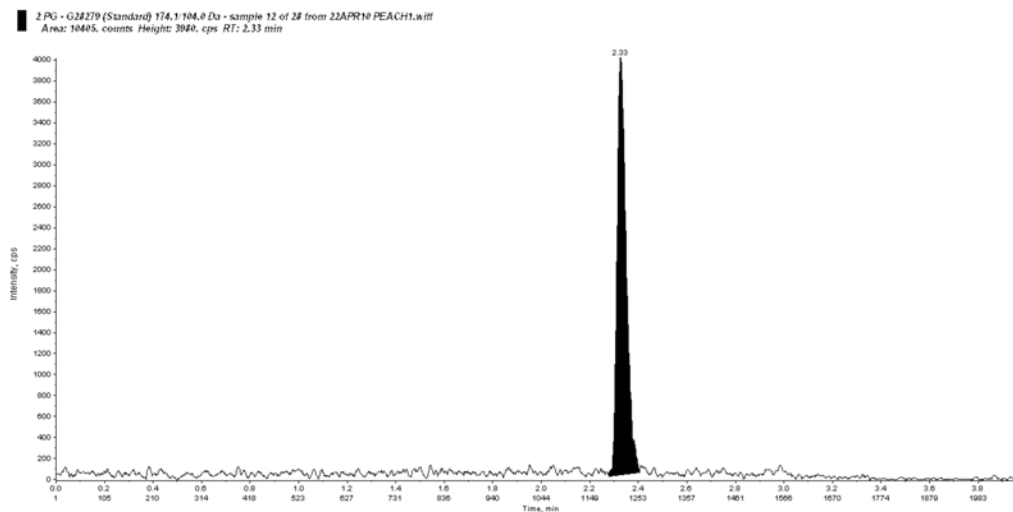


1. Standard (1.0 pg/ μ L), 10 μ L injection volume, 10.0 pg of G28279 injected, peak area = 52816, from analysis of peach.

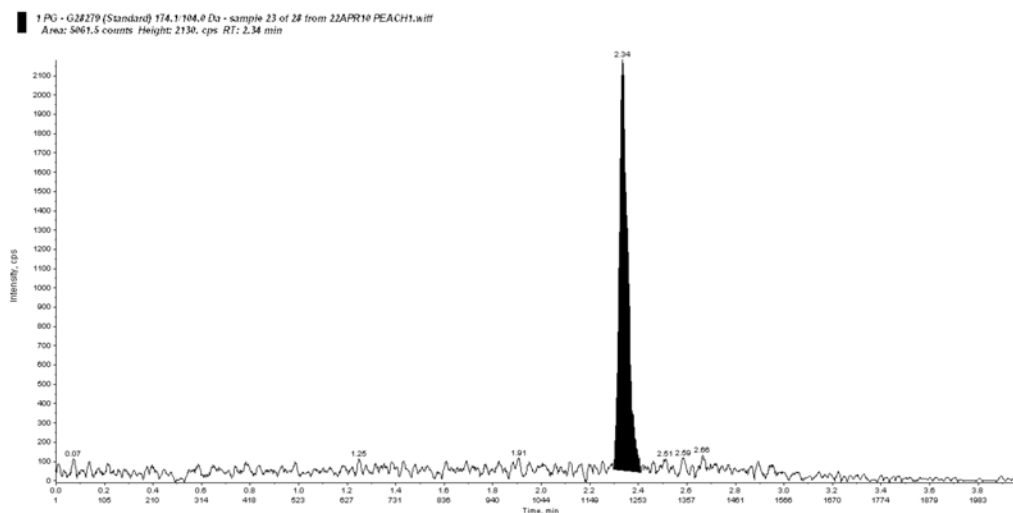


2. Standard (0.50 pg/ μ L), 10 μ L injection volume, 5.0 pg of G28279 injected, peak area = 28593, from analysis of peach.

**FIGURE 9 Representative Chromatograms – G28279 Calibration Standards.
Transition $m/z = 174.1 \rightarrow 104.0$ (Continued)**

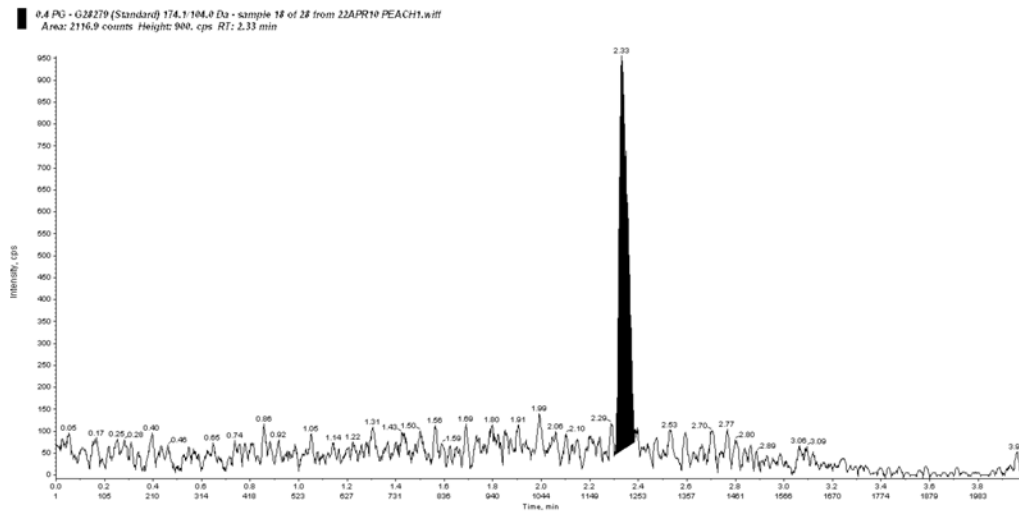


3. Standard (0.20 pg/ μ L), 10 μ L injection volume, 2.0 pg of G28279 injected, peak area = 10405, from analysis of peach.



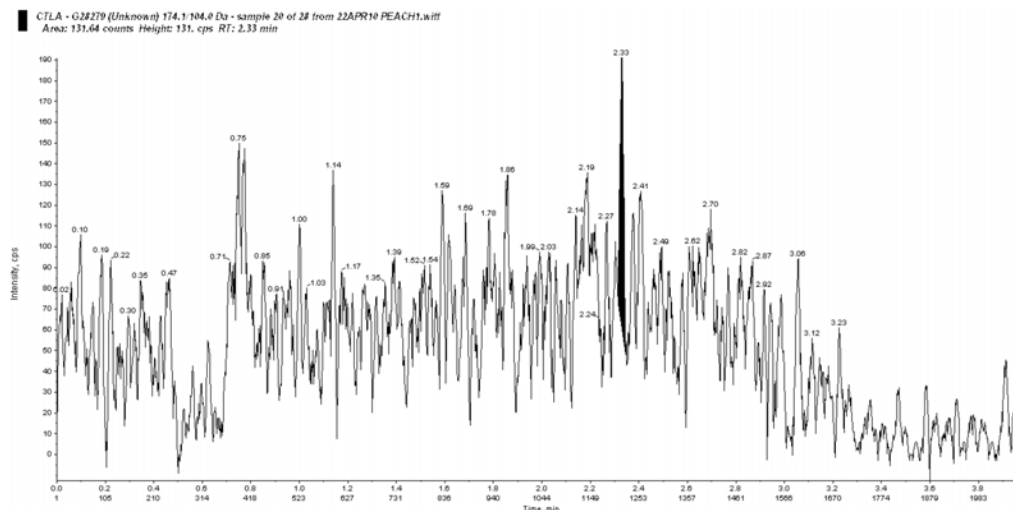
4. Standard (0.10 pg/ μ L), 10 μ L injection volume, 1.0 pg of G28279 injected, peak area = 5062, from analysis of peach.

**FIGURE 9 Representative Chromatograms – G28279 Calibration Standards.
Transition $m/z = 174.1 \rightarrow 104.0$ (Continued)**

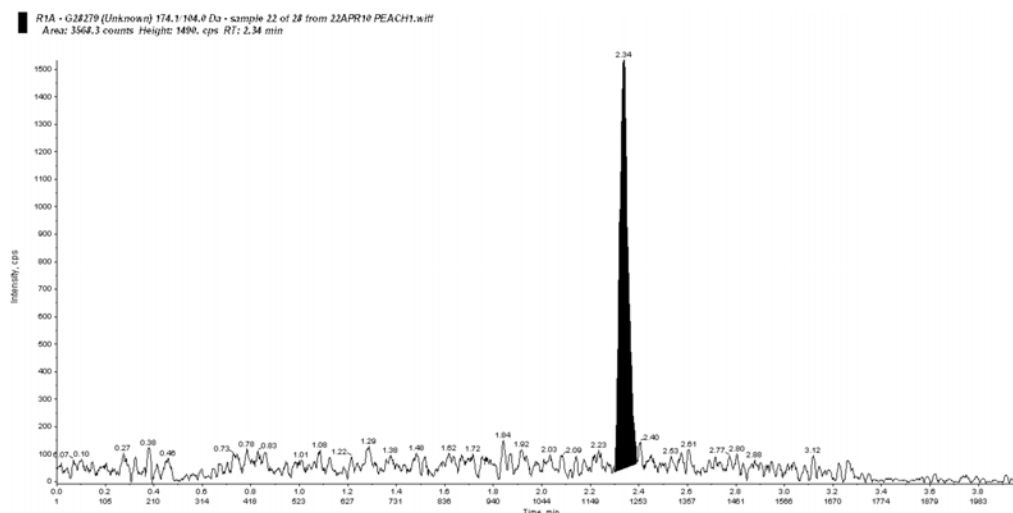


5. Standard (0.05 pg/ μ L), 8 μ L injection volume, 0.40 pg of G28279 injected, peak area = 2117, from analysis of peach.

FIGURE 10 **Representative Chromatograms – G28279 Peach Transition m/z = 174.1→104.0**

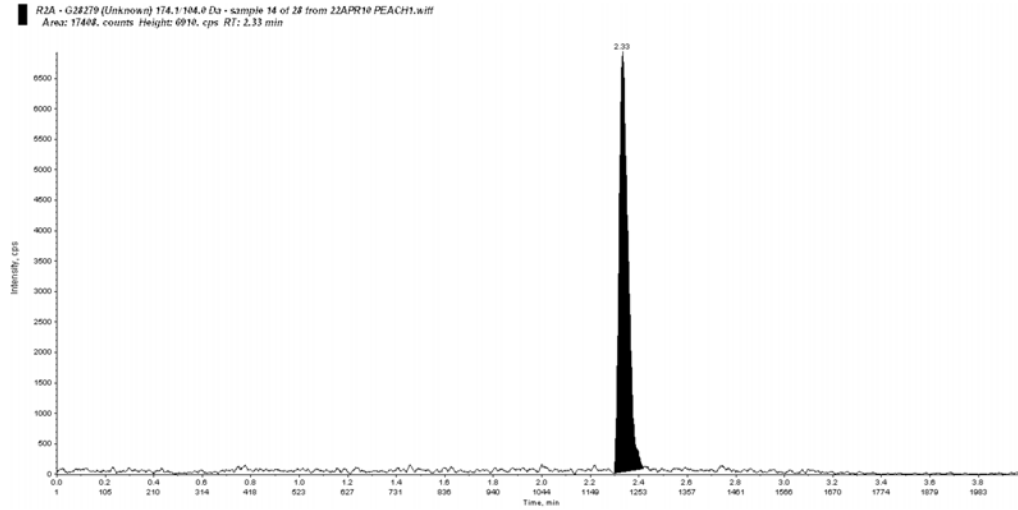


1. Peach Control, <0.01 ppm determined, Set 22APR10 PEACH1.

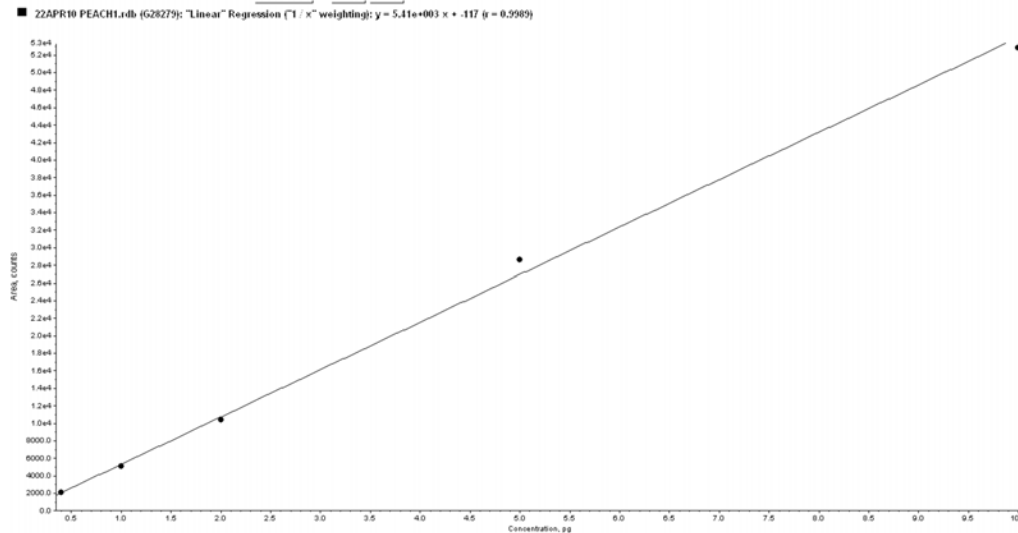


2. Peach Control + 0.01 ppm R1A, 96% Recovery, Set 22APR10 PEACH1.

FIGURE 10 **Representative Chromatograms – G28279 Peach Transition m/z = 174.1→104.0 (Continued)**

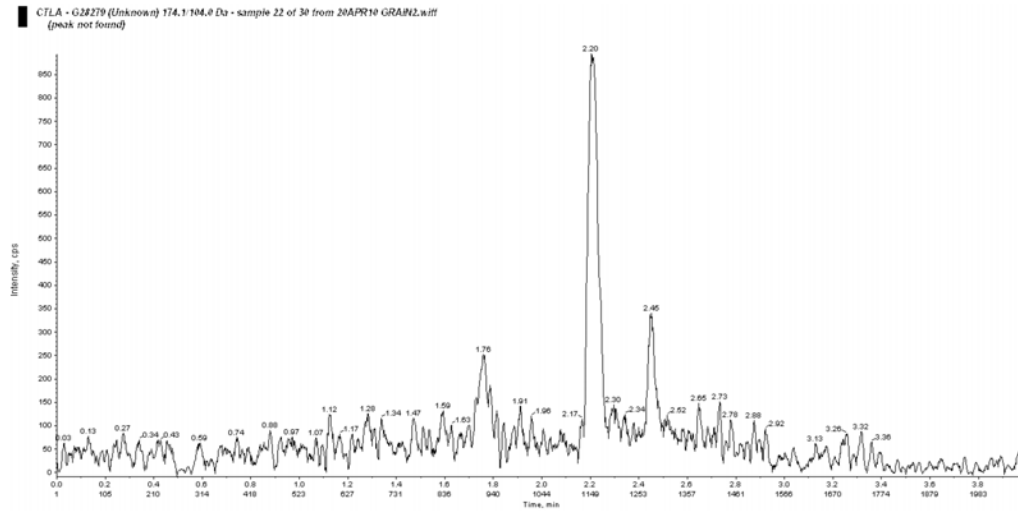


3. Peach Control + 0.10 ppm R2A, 99% Recovery, Set 22APR10 PEACH1.

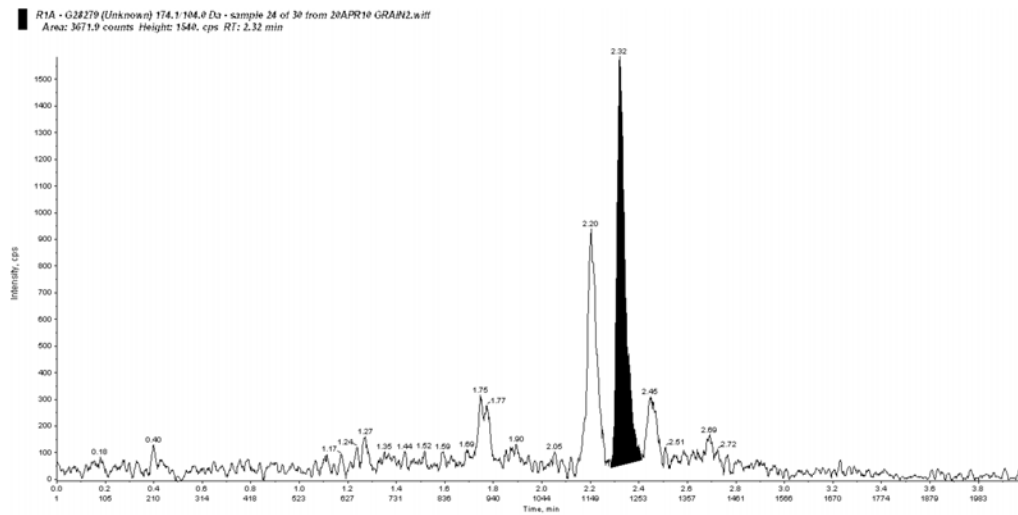


G28279 Peach Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of G28279 standards.

FIGURE 11 **Representative Chromatograms – G28279 Corn Grain**
Transition $m/z = 174.1 \rightarrow 104.0$

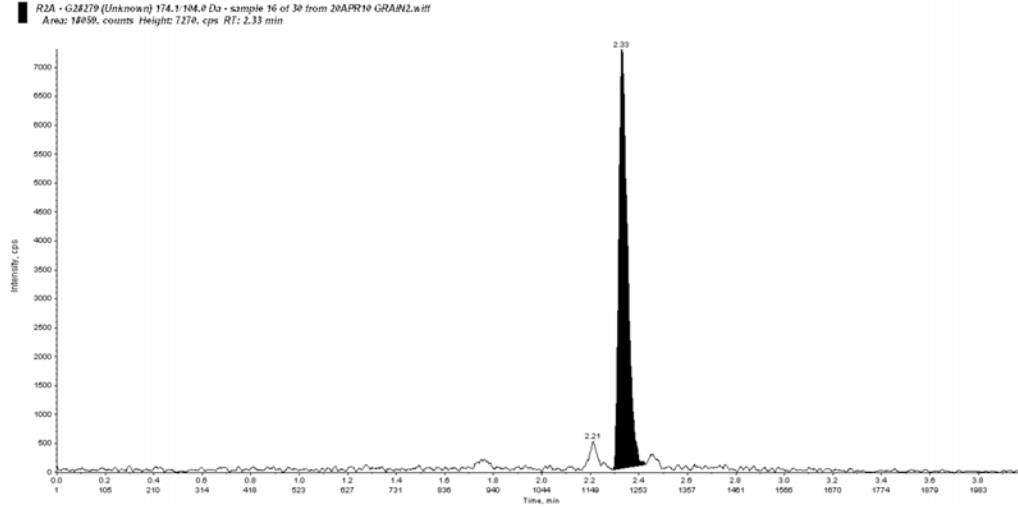


1. Corn Grain Control, <0.01 ppm determined, Set 20APR10 GRAIN2.

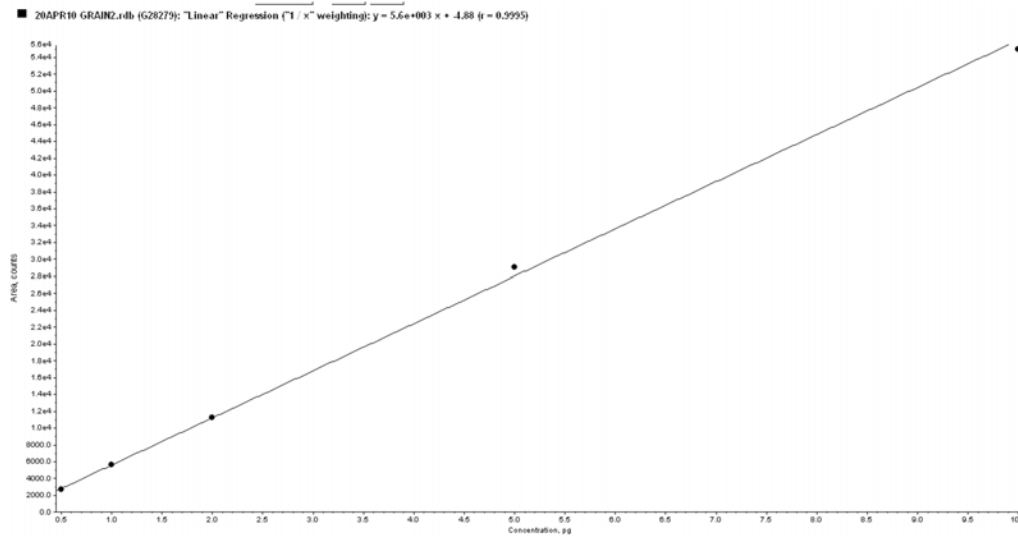


2. Corn Grain Control + 0.01 ppm R1A, 99% Recovery, Set 20APR10 GRAIN2.

FIGURE 11 **Representative Chromatograms – G28279 Corn Grain**
Transition $m/z = 174.1 \rightarrow 104.0$ (Continued)

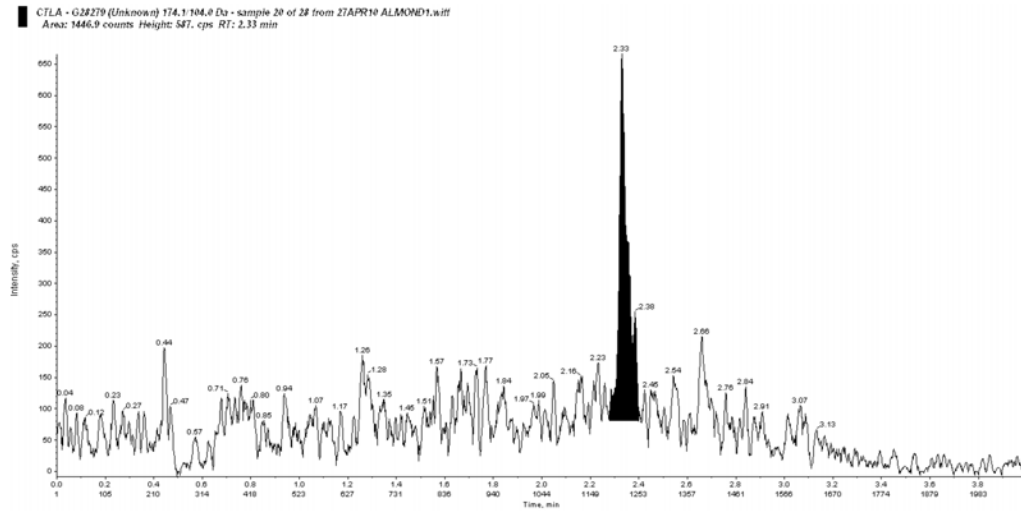


3. Corn Grain Control + 0.10 ppm R2A, 97% Recovery, Set 20APR10 GRAIN2.

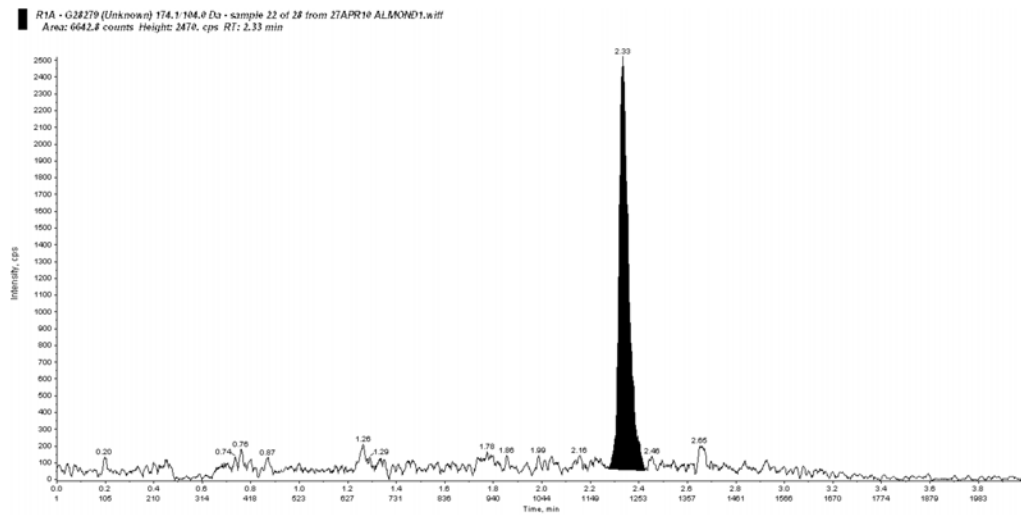


G28279 Corn Grain Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of G28279 standards.

FIGURE 12 **Representative Chromatograms – G28279 Almond Transition**
 $m/z = 174.1 \rightarrow 104.0$

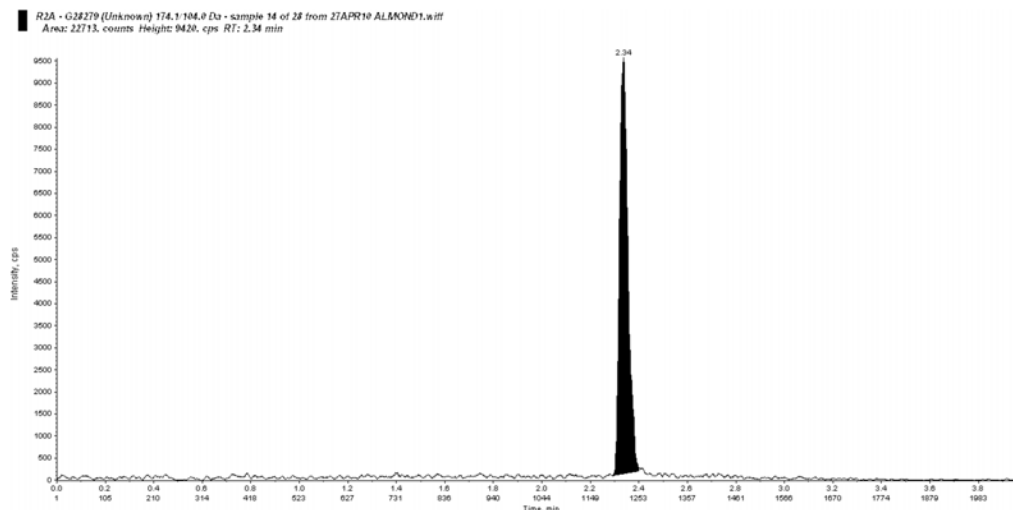


1. Almond Control, <0.01 ppm determined, Set 27APR10 ALMOND1.

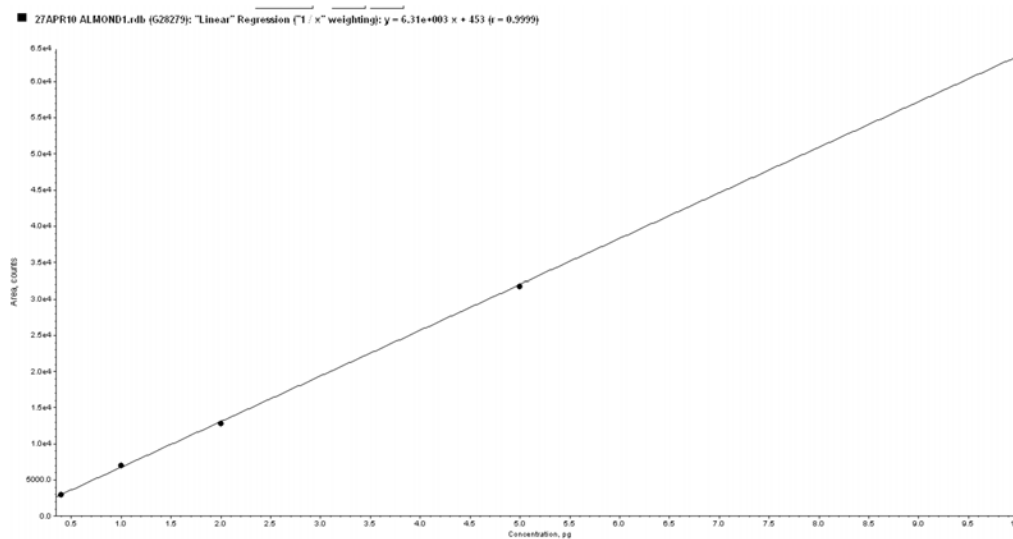


2. Almond Control + 0.01 ppm R1A, 114% Recovery, Set 27APR10 ALMOND1.

FIGURE 12 **Representative Chromatograms – G28279 Almond Transition**
 $m/z = 174.1 \rightarrow 104.0$ (Continued)



3. Almond Control + 0.10 ppm R2A, 103% Recovery, Set 27APR10 ALMOND1.



G28279 Almond Standard Curve: Y-axis represents LC-MS/MS peak area, and X-axis represents the injected amount (pg) of G28279 standards.

APPENDICES SECTION

APPENDIX 1 Apparatus

US suppliers

- 1.0 Balance, top loading, capable of actually weighing 10.00 grams, Mettler PB3002-S, or equivalent.
- 2.0 Condenser, Allihn, 30-cm jacket, Fisher Cat #97-734A, or equivalent.
- 3.0 Heating mantle, 500 mL.
- 4.0 Round bottom Flask with 24/40 ground glass joint, 500 mL.
- 5.0 Glass amber Boston Round bottles, 250mL, Fisher Cat# 02-911-900, or equivalent.
- 6.0 Reeve Angel Filter, 802 Fluted, Cat# 5802-240.
- 7.0 Vial for HPLC injection, Wheaton 2-mL glass, or equivalent.
- 8.0 Autopipettors Eppendorf Research Model, or equivalent.
- 9.0 Disposable Pasteur pipettes with 2-mL bulb.
- 10.0 Graduated cylinders, 10, 100, 1000-mL.
- 11.0 Volumetric flask, 50, 100-mL.
- 12.0 Polypropylene centrifuge tubes, 15-mL and 50-mL, Becton Dickinson and Company, or equivalent.
- 13.0 Whatman Filter, 2V, Cat# 1202-240.

APPENDIX 2 Reagents

US suppliers

- 1.0 Concentrated formic acid, 90%, Laboratory Grade, Fisher Scientific Cat. #A-119-1, or equivalent.
- 2.0 Water (H₂O), deionized and purified in-house with a HYDRO™ purification system or equivalent.
- 3.0 Methanol (MeOH), HPLC Grade, Fisher Scientific Cat. #452SK-4, or equivalent.

Preparation of reagents

- 1.0 80/20 Methanol:Water (v/v) – prepared by mixing 800 mL of methanol with 200 mL of water.
- 2.0 90/10 + 0.1% formic acid Water:Methanol (v/v) – prepared by mixing 900 mL of water with 200 mL of methanol and 1mL of concentrated formic acid.
- 3.0 Mobile Phase A: 0.1% formic acid aqueous solution– prepared by mixing 1.0 mL of concentrated formic acid to 1L of water.
- 4.0 Mobile Phase B: 0.1% formic acid in Methanol– prepared by mixing 1.0 mL of concentrated formic acid to 1L of Methanol.

APPENDIX 3 Method Flowchart

