

Analytical Method for Glyphosate and AMPA in Raw Agricultural Commodities Using LC/MS/MS

Overview

Purpose & Scope

This SOP describes the analytical method used by Environmental Sciences Technology Center personnel to determine trace quantities of glyphosate (N-phosphonomethyl glycine) and AMPA (aminomethylphosphonic acid) in RACs.

Method Summary

Glyphosate and AMPA are isolated from crop matrices by high speed blender extraction using 0.1% formic acid in water and methylene chloride. Following centrifugation, an aliquot of aqueous phase extract is treated with a solid phase extraction cleanup. The analytes are analyzed by LC-MS/MS and quantitated using internal standards. The working range of the method is from 0.05 ppm (LLMV) to 6.0 ppm of glyphosate and AMPA. The method has been validated for the analysis of the raw agricultural commodities of corn, soybeans, canola, cotton, sugar beets, alfalfa, citrus and cotton oil.

Safety Precautions

Follow current Monsanto safety policies. Important precautions include:

- Some solvents are volatile and flammable. Care must be taken to keep them away from any source of ignition.
- Ensure proper ventilation to avoid excessive exposure to solvent vapors.
- Read and follow all safety warnings on reagent containers.

Abbreviations

The following abbreviations are used in this SOP:

Abbreviation	Definition
AMPA	aminomethylphosphonic acid
amu	atomic mass units
ARS	analytical reference standard
DI	Deionized water (Milli-Q water or equivalent)
ESI	electrospray ionization
g	gram
LC/MS/MS	high performance liquid chromatography/tandem mass spectrometry
LLMV	lower limit of method validation
MeCl ₂	Methylene Chloride
MeOH	methanol
µg	microgram
mL	milliliter
mm	millimeter
MRM	multiple reaction monitoring
ms	millisecond
MS	mass spectrometry
RAC	raw agricultural commodity
ppm	parts-per-million
SPE	solid phase extraction
SOP	standard operating procedure
V	volts

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Materials

Equipment

The following equipment is used in this procedure. Specific brands are listed to aid the analyst in finding items. In most cases, equivalent equipment from other vendors can be used.

Equipment	Number/Specification
Balances (electronic): • Analytical • Top loading	Mettler: • AE 240 • PM 4800
Plastic Solution Containers	Nalgene, 4 oz. HDPE, No. 2018 0125
Blender (motor / glass container)	Waring™ No. 7010G
Centrifuge Bottles (250 mL)	Nalgene, PPCO with sealing cap Nalgene No. 3141-0250
Centrifuge (superspeed automatic refrigerated)	Sorvall Instruments (DuPont Co.), Model RC6+
Multi-Channel SPE System	Cerex System 96-II ,SPE/ware, Inc.
HPLC System	Agilent 1200 Components, Solvent Degasser, 2 Binary Pumps, Autosampler, Column Compartment
HPLC Column	Bio-Rad Fast Acid Column Cat. No. 125-0100
Alternate HPLC Column	Bio-Rad Cation H, Cat. No. 125-0129
Mass Spectrometer	Applied Biosystems PE Sciex API 5000™ with Turbo-V atmospheric pressure ionization source
Data Acquisition System	Windows XP based workstation with PE Sciex Analyst® software
Dispensers : 100 mL	Brinkmann :No. 22-22-050-1
Sample vials: (2-20 ml)	glass or plastic with closures. Wheaton Cryule Vial No. 985746
Volumetric flasks:100 mL	Kimax Class A, No. 28014P 100
Pasteur pipettes: 5.75" and 9"	Fisher No.: 13-6678-6A and 13-6678-6B
Graduated cylinders (1 L)	suitable for procedure
Autopipettes: (500 µL, 1000 µL, 100 µL– 1000 µL, 1-5 mL, 2-10 mL)	suitable for procedure
Solid phase extraction	Strata-X, 96 well, 60mg, Phenomenex No. 8E-S100-UGB
Sample collection plate	96 well, 1 mL, polypropylene Agilent No. 5042-1387
Sample collection plate closing mat	Agilent No. 5042-1389
Syringe (disposable with Luer-Lok tip, 10 mL)	Becton-Dickinson, VWR Cat. No. BD305462
Syringe filter (25 mm disposable filter device, 0.45 µm pore size)	Whatman® GD/XP No. 6994-2504

Transformer (variable, used to control blender speed)	VWR Cat. No. 62546-048
Water purification system	Millipore Compact Milli-Q Plus

**Chemicals &
Reagents**

The following reagents are used in this method. **Note:** Specific brands are listed, but in most cases, equivalent reagents from other vendors can be used. It is important to use high quality reagents to avoid chromatographic interferences. It is recommended that the isotopic purities of the internal standard materials (^{13}C , ^{15}N -glyphosate and D_2 , ^{13}C , ^{15}N -AMPA) be verified prior to use.

Chemical/Reagent	Number/Specification
AMPA, (Aminomethylphosphonic acid)	Monsanto ARS Program (>95%)
Glyphosate (N-phosphonomethyl glycine)	Monsanto ARS Program (>95%)
$^{13}\text{C}_3$, ^{15}N -Glyphosate	Monsanto ARS Program
D_2 , ^{13}C , ^{15}N -AMPA	Monsanto ARS Program
Deionized water	Milli-Q water or equivalent
Formic Acid(98% min.)	EMD Cat. No. EM-FX0440-7
Acetonitrile, HPLC grade	Burdick & Jackson Cat. No. 015-4
Methylene Chloride, HPLC grade	JT Baker, VWR Cat. No. JT9315-3
Methanol, HPLC grade	Burdick & Jackson Cat. No. 230-4

Reagent/Solution Preparation**Procedure**

Prepare the following reagent solutions for use in sample analysis. The absolute volume of the solutions may be varied at the discretion of the analyst, as long as the correct proportions of the components are maintained. Assign a six month expiration date to these solutions unless a shorter expiration is specified on the label.

Solution	Preparation
HPLC Mobile Phase A and Extraction Solvent:	0.1% Formic acid in water Add 1.0 mL Formic acid to 1 L of DI water.

Standards Preparation

Overview All standard calibration and fortification solutions must be properly labeled and stored in polypropylene bottles with airtight lids at <10 °C. Preparation procedures which result in equivalent solutions may be substituted. Various additional solutions may be prepared.

Stability Data in the Monsanto archives documents the stability of solutions from 0.05 to 1000 ppm of glyphosate and AMPA prepared in DI water for 23 months when stored at <10 °C. Solutions prepared in 0.1% formic acid/water demonstrated stability for 99 days at <10 °C.

Glyphosate and AMPA Stock Solutions (1000 µg/mL) Weigh and dissolve 0.1000 ±0.0005 g (weight adjusted for purity) of glyphosate standard in 100 mL of DI water in a volumetric flask. This stock solution contains 1000 µg/mL of glyphosate. The solution should be sonicated briefly to ensure complete dissolution.

In a similar manner, dissolve 0.1000±0.0005 g (weight adjusted for purity) of AMPA standard in 100 mL of DI water in a volumetric flask. This stock solution contains 1000 µg/mL of AMPA. The solution should be sonicated briefly to ensure complete dissolution.

Mixed Stock Standard Solutions Prepare the following mixed standards by dilution of the appropriate stock or mixed solution with DI water in volumetric flasks. These solutions will be used for the preparation of working solutions and fortification solutions.

Mixed Standard Solution Prepared Conc. (µg/mL)	Volume to Dilute (mL)	Mixed Solution To Dilute (µg/mL)	Final Volume (mL)
100	10.0 of each	1000 Glyphosate 1000 AMPA	100
10.0	10.0	100.0	100
1.0	10.0	10.0	100

Internal Standard Stock Solutions Weigh and dissolve 0.0100 ±0.0005 g each of ¹³C₃¹⁵N-Glyphosate and D₂¹³C¹⁵N-AMPA in 100 mL of DI water in separate volumetric flasks. Each stock solution contains 100 µg/mL of internal standard. The solutions should be sonicated briefly to ensure complete dissolution.

Internal Standard Working Solution (Mixed) Pipette 0.5 mL of each of the 100 µg/mL stock solutions of ¹³C₃¹⁵N-glyphosate and D₂¹³C¹⁵N-AMPA into a single 100 mL volumetric flask and dilute to the mark with DI water. This stock solution contains 0.5 µg/mL each of ¹³C₃¹⁵N-glyphosate and D₂¹³C¹⁵N-AMPA.

**Glyphosate/
AMPA Standard
Solutions**

The preparation of working calibration solutions is given below. Add the volume of the appropriate mixed stock solution to a 100 mL volumetric flask and dilute to volume with 0.1 % formic acid in water. Additional standard levels may be prepared as necessary. These standard solutions will be mixed with internal standards for each analytical set (see Sample Preparation Procedure). Each standard level and sample will contain 0.05 µg/mL of the internal standards ($^{13}\text{C}_3$, ^{15}N -glyphosate and D_2 , ^{13}C , ^{15}N -AMPA).

To Prepare Working Solution (µg/mL)*	Add (mL) Mixed Stock	Mixed Stock Conc. (µg/mL)	Final Volume (mL)
0.0025	0.25	1.0	100
0.005	0.50	1.0	100
0.010	1.0	1.0	100
0.030	3.0	1.0	100
0.060	6.0	1.0	100
0.10	1.0	10.0	100
0.30	3.0	10.0	100
0.60	6.0	10.0	100

*µg/mL of glyphosate and AMPA.

**Fortification
Solutions**

The preparation of standard solutions used to fortify quality control samples is shown below. Aliquot the appropriate volume and concentration of the mixed or separate stock solution into a 100 mL volumetric flask. Dilute to volume with deionized water and mix well. Additional mixed or separate solutions may also be prepared as needed in a similar manner.

To Prepare Fortification Solution (µg/mL)	Add (mL) Stock Solution	Stock Solution Conc. (µg/mL)	Final Volume (mL)
0.5	5.0	10 (mixed)	100
5.0	5.0	100 (mixed)	100
50.0	5.0 of each	1000 Glyphosate 1000 AMPA	100

Example fortification: to prepare a 0.50 ppm QC sample, add 1.0 mL of 5.0 ug/mL fortification solution to 10.0 g of control sample material.

Sample Preparation Procedure

Raw Sample Preparation

Raw sample material must be thoroughly ground and homogenized prior to analysis. To process the raw samples, add dry ice (ca. 25% w/w) to the frozen sample and chop with a Hobart chopper, or equivalent high-speed homogenizer. Place the samples in a -20 °C freezer overnight to allow sublimation of the dry ice prior to weighing.

Sample Extraction and Cleanup

The following describes the preparation of samples for analysis by LC/MS/MS. A typical extraction set will include study samples, QC samples and standards.

Step	Action
1	Weigh 10.0 g \pm 0.1 g of sample into a blender jar.
2	If required, fortifications must be made at this stage by adding the correct volume of the appropriate fortification standard solution to the designated control samples.
3	Add 100 mL of methylene chloride and 100 mL of 0.1% formic acid in water. Blend using a Waring blender at high speed for about 2 minutes. The MeCl ₂ serves as a cleanup solvent. Analytes will reside in the aqueous layer.
4	Transfer the blended sample to a 250 mL centrifuge bottle. Do <u>not</u> rinse the blender jar. Balance pairs of centrifuge bottles by adding methylene chloride to the lighter bottle until its weight is within 0.5 g of the heavier bottle.
5	Centrifuge at about 1,000 rpm for 20 minutes in a refrigerated centrifuge.
6	Filter and transfer about 10 mL of the aqueous layer of the sample into a suitable sample vial using a 10 mL syringe with 25mm x 0.45 μ M filter. The sample may be stored in a freezer at this point. Discard the remaining aqueous, MeCl ₂ layer and sample solids.
7	Pipette 0.2 mL of the working internal standard into a sample vial (fresh vial).
8	Pipette 1.8 mL of the aqueous layer collected in Step 6 above into the sample vial containing the internal standard and mix well.
9	Place a 96 well SPE plate on a 96 well collection plate. Add about 0.45 mL of each sample/internal standard mixture or working calibration standard (as prepared below) to a well in the SPE plate.
10	Place the SPE plates on a "Multi-Channel SPE" processor and apply gas pressure to force the liquids through the sorbent bed into the collection plate. Remove the collection plate and apply the closing mat. The samples are ready for LC/MS/MS analysis.

Working Standard Preparation

Prepare the working calibration standards and samples in the same manner.
Prepare a solution of 9 parts working standard and 1 part internal standard (e.g., combine 1.8 mL of each calibration solution with 0.2 mL of 0.50 ug/mL mixed internal standard solution and mix well). Process a set of standards for each extraction set as described in steps 9 and 10 above.

Extract Dilution High level samples producing a response ratio (or analyte response) greater than the highest standard of the calibration curve, must be diluted to within the standard range and reanalyzed.

Dilutions must be made prior to addition of the internal standards. Dilute an aliquot of the extract with 0.1% formic acid in water then add the internal standard as described in the sample preparation procedure (i.e., combine 1.8 mL of dilute sample plus 200 μ L of working internal standard). **Note:** The diluted sample may be analyzed in any chromatographic set, provided that the set contains control and fortified control samples and meets the other requirements.

Instrumental Analysis

Overview The requirements for sample analyses include but are not limited to the following:

- An extraction set is a group of study samples, QC samples and calibration standards prepared together.
- QC samples are paired fortified/non-fortified samples. The non-fortified sample (control) will generally be a prescreened low background level study sample serving both as a study sample and a QC sample. At least one pair of fortified and non-fortified samples will be prepared for every extraction set.
- A chromatographic set is a group of unknown samples, the associated QC samples and calibration standards undergoing instrumental analysis together. Each chromatographic set will contain at least one pair of fortified and non-fortified samples.
- Analyte calibration must be performed for each chromatographic set using a calibration curve with a minimum of five calibration levels. The chromatographic set must begin and end with a calibration standard (i.e., control samples, fortified samples and field samples bracketed by calibration standards). The response for all fortified QC and treated samples greater than or equal to the LOQ must fall within the range of calibration standard concentrations. The concentrations of the calibration standards will be randomized throughout the chromatographic set. The average accuracy at each standard level must be within 70-120%.
- The average analytical recovery of fortified samples for each analyte in a set must range between 70-120% of the amount fortified.

Instrument Setup

Instrument operation is controlled by acquisition methods containing all HPLC, source interface, and mass spectrometer operating parameters. The typical precursor and product ions for the analytes are shown below along with ions for possible use in confirmatory analyses. Alternate ions may be used if they provide better data (sensitivity and/or specificity). The following equipment and conditions are instrument dependent and may be modified to obtain optimal instrument performance and maximize sensitivity. Actual method parameters must be documented in the raw data.

The following equipment and conditions were used during method validation.

LC-MS/MS System Conditions for Analysis of Glyphosate and AMPA					
Agilent 1200 HPLC System and AB/Sciex API 5000 Extra HPLC Pump for Post Column Solvent Addition Column: Bio-Rad Fast Acid 100 mm x 7.8 mm, 9 μ Guard Column: none Injection Volume: 40 μ L Autosampler Temp.: 4 °C Column Temp.: 22 °C Split Ratio:~1:1 Mobile Phase A: 0.1% formic acid in H ₂ O Mobile Phase B: None Evaporation Solvent Tee'd Post Column : MeOH at 700 μ L/min. Flow Rate: 1500 μ L/minute Isocratic: <ul style="list-style-type: none"> Initial: 100 % A 16 minutes: 100 % A 					
Mass Spectrometer Conditions					
Scan Type: Negative Ion MRM Resolution Q1: Unit Resolution Q3: Unit Ion Source: ESI					
Duration : 16 min. Curtain Gas (CUR): 15 Collision Gas (CAD): 6 Gas 1: 40 Gas 2: 30			IonSpray Voltage (IS): -4500 V Entrance Potential: -10 Interface heater: On Temp: 600 °C Scan Time (ms): 200		
Analyte:	Precursor Ion Q1 (amu)	Product Ion Q3 (amu)	DP (V)	CE (V)	CXP (V)
Glyphosate	168	63	-70	-31	-25
Gly (IS)	172	63	-70	-31	-25
AMPA	110	63	-70	-30	-20
Ampa (IS)	114	63	-70	-30	-20
Confirmatory Ions					
Glyphosate	168	79	-70	-50	-31
Gly (IS)	172	79	-70	-50	-31
AMPA	110	79	-70	-40	-20
Ampa (IS)	114	79	-70	-40	-20

When confirmatory or alternate ions are used, they must be appropriately named in the acquisition method to preclude confusion in data reporting (e.g., Gly_C or Gly_79 for glyphosate confirmatory ion).

Alternate HPLC Column and Conditions

Certain matrices, especially canola seed and alfalfa hay provided more consistent results using a different HPLC column. The alternate column allows the use of an organic modifier in the mobile phase and requires no split or addition of evaporation solvent.

LC-MS/MS System Conditions for Analysis of Glyphosate and AMPA

Column: Bio-Rad Cation-H Guard Column 30 mm x 4.6 mm

Injection Volume: 10 μ L

Autosampler Temp.: 4 $^{\circ}$ C

Column Temp.: 50 $^{\circ}$ C

Split Ratio: none

Mobile Phase A: 0.1% formic acid in H₂O

Mobile Phase B: Acetonitrile

Flow Rate: 500 μ L/minute

Isocratic:

- Initial: 80 % A 20% B
- 16 minutes: 80 % A 20%B

Mass Spectrometer Conditions : Same as Above

**Data
Processing**

Process the data using the Analyst™ quantitation wizard. A method may be created which processes the data for the MRM transition pairs established in the acquisition method. The method detects and integrates the analyte peaks based on retention time and MRM transition. Chromatograms may be smoothed prior to integration, as long as the smoothing is consistent throughout the entire sample set. Manual peak integration should be used when the automated procedure is not effective due to baseline noise. The sample weights and dilution factors required to complete the calculations must be added during data processing if not input prior to the start of the instrument run. The data processing parameters must be documented in the raw data.

Calculations

Analyte concentrations are calculated using the Analyst® software. The software calculates the standard curve and applies the dilution factor to account for sample weight and dilution volume. Linear or quadratic calibration curves may be used for quantitation. All the samples from a study must be analyzed with the same type of calibration curve for a given analyte.

**Linear
Calibration
Curve**

The Analyst® software automatically derives the calibration curve using the area response ratios (y) versus the concentration ratios (x) of the external and internal standards for all standards injected with the chromatographic set. A weighted linear regression (1/x) standard curve is used. The resulting equation defining the standard curve is shown below:

$$y = Ax + B \text{ where,}$$

x = concentration injected (µg/L)

y = detector response ratio (peak area ratio)

The values of coefficients A and B, the slope and the y intercept, respectively, are estimated using a linear least squares regression.

The calculation may be checked manually by rearranging the curve determined for the linear regression to the form shown below then applying correction for sample weight and final volume.

$$\frac{y - B}{A} = x \quad \text{or} \quad \frac{[\text{sample response} - y \text{ intercept}]}{\text{slope}} = \text{concentration injected}$$

**Quadratic
Calibration
Curve**

The Analyst® software automatically derives the calibration curve using the area response ratios (y) versus the concentration ratios (x) of the external and internal standards for all standards injected with the chromatographic set. A weighted quadratic curve (1/x) is used. The resulting equation defining the standard curve is shown below:

$$A (\mu\text{g/mL analyte})^2 + B (\mu\text{g/mL analyte}) + C = PKR_{\text{analyte}} \text{ where,}$$

PKR_{analyte} is the detector response ratio (peak area ratio)

A, B and C are curve constants

The calculation may be checked manually by applying the solution for quadratic equations as shown below then applying correction for sample weight and final volume. (**Note:** Subtract the response PKR_{analyt} from C first.)

$$\text{Injected Concentration X } (\mu\text{g/mL analyte}) = \frac{-b \pm \sqrt{(b^2 - 4ac)}}{2a}$$

**Analyte
Concentration**

The Analyst™ system automatically calculates the raw concentration of the injected sample relative to the standard curve (*calculated concentration* (µg/mL)). This value is also automatically multiplied by any value entered in the *dilution factor* column.

The analyte concentration in the raw sample may be calculated using the following information for typical samples:

- A) Sample weight: 10.0 g
- B) Sample extract volume: 100 mL (initial 100 mL aqueous extraction solvent).

Therefore, to correct the *calculated concentration* for final volume and sample weight, enter the volume and weight as a single factor (v/wt) in the *dilution factor* column.

Example: Sample with an injected concentration of 0.05 µg/mL and dilution factor of 10 (100 mL/10.0 g) is displayed in the *calculated concentration* column as 0.50 ppm.

$$\begin{aligned}\text{ppm analyte found} &= [\text{calculated concentration (}\mu\text{g/mL)} \times (\text{B})] / (\text{A}) \\ &= [(0.05 \mu\text{g/mL})(100 \text{ mL})] / 10 \text{ g} = 0.50 \text{ ppm}\end{aligned}$$

Any additional dilutions due to responses out of standard curve range must also be incorporated into the *dilution factor* column. For a 10x dilution, the factor entered for the sample above would be 100.

**Fortified
Sample
Recovery**

Fortified sample recoveries must be determined after subtraction of any background analyte ppm found in the unfortified control sample.

$$\% \text{ recovery} = \frac{(100) (\text{ppm found} - \text{ppm in control})}{\text{ppm added}}$$

Documentation

The analytical raw data packages will include (as a minimum): the sample worksheet, instrumental sample list, calibration curves, MRM chromatograms, result table, instrument acquisition and processing parameters. The data will be processed and printed following MS data acquisition.

Interferences

Analyte response suppression due to the sample matrix has been minor, however the use of internal standards compensates for any difference in response between samples and standards. Sample carry over has been observed with some autosamplers and can be a problem for low level samples following injection of high level samples. Blank vials may be needed between samples to eliminate carry over.

Column Maintenance

The performance of the HPLC column may deteriorate over time as shown by poor peak shape or sensitivity. If the alternate column (Bio-Rad 30 X 4.6 mm Cation H) is used, it may be economically replaced as necessary, to maintain performance. The larger Bio-Rad Fast Acid 100 X 7.8 mm column may be regenerated using the procedure provided by the manufacturer to restore column performance.

Example Chromatograms

Example LC/MS/MS chromatograms for calibration standards and control and fortified corn stover samples are provided in Appendix B. Chromatograms are shown for the quantitation and confirmation ions of each analyte using the 30 X 4.6 mm Bio-Rad Cation H column.

Method Validation Results

The analytical method was validated for the analysis of the raw agricultural commodities of corn, soybeans, cotton, sugar beets, canola, alfalfa, oranges and cotton oil. The method may also be used for soybean oils with additional validation analyses. The validation tested:

- the dynamic range, precision, accuracy of the calibration standards.
- method precision and accuracy for sample analyses.
- sample dilution method for high level samples.
- storage stability of standards and sample extracts.

The calibration standards were evaluated by injecting three replicates of each standard level, including 0.0025, 0.005, 0.10, 0.03, 0.06, 0.10, 0.6 ug/mL in the course of a large set of corn stover samples. The standards were processed as one combined curve for each analyte. The curves were analyzed using quadratic regression with 1/x weighting. The percent accuracies were between 98 and 101 percent of expected values and the percent RSD at each level was < 5 percent.

Sample recovery and precision was tested at 0.05 ppm (LLMV) and at levels from 0.5 to 400 ppm depending on the matrix. Average recoveries at each test level were between 78 and 109 percent of expected values for all matrices. The percent RSD at each test level was between 0.5 and 14 percent. The dilution method for out of range samples provided recovery and precision results equivalent to undiluted samples.

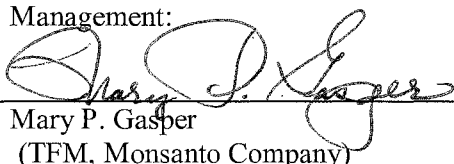
Stability was established for final sample extracts maintained refrigerated (<10 °C) during an analysis period of up to 7 days. Data was developed to support establishment of a common storage stability period of 30 days for initial sample extracts stored frozen (-20 °C). The stability of analytes in working calibration standard solutions stored at <10 °C was demonstrated over a period of 99 days. An alternate HPLC column was identified and confirmed for analysis all matrices.

References

Glyphosate and AMPA Residue Determination in Crops
Glyphosate and AMPA / Crops / GW / 06 / 2
Agrisearch UK Limited
(Independent Laboratory Validation , Monsanto Study 07-63-R3)

Author(s) / prepared by: J. Mark Allan and Ronald K. Beasley

Management:



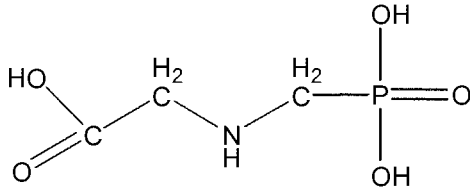
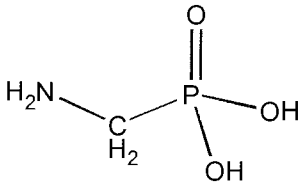
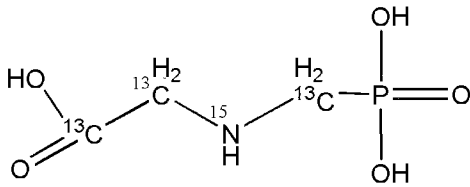
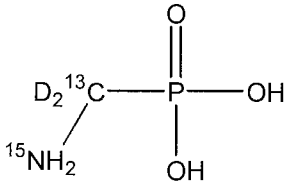
Mary P. Gasper

(TFM, Monsanto Company)

Date:

9 / 29 / 2010

Appendix A: Chemical Structures

	<p>Glyphosate</p> <p>N-phosphonomethyl glycine</p> <p>Molecular weight: 169.07 Exact mass: 169.01</p>
	<p>AMPA</p> <p>Aminomethylphosphonic acid</p> <p>Molecular weight: 111.04 Exact mass: 111.01</p>
	<p>¹³C₃ ¹⁵N-Glyphosate</p> <p>[¹³C₃, ¹⁵N] N-(phosphonomethyl)glycine</p> <p>Molecular weight: 173.04 Exact mass: 173.02</p>
	<p>D₂ ¹³C ¹⁵N-AMPA</p> <p>[13-C, 15N, methylene-D₂] aminomethylphosphonic acid</p> <p>Molecular weight 115.04 Exact mass: 115.02</p>

Appendix B: Representative Chromatograms

MRM Chromatograms for Glyphosate Quantitative Ion in Corn Stover

Sample Id.: Calibration Standard 0.005 ug/mL Glyphosate

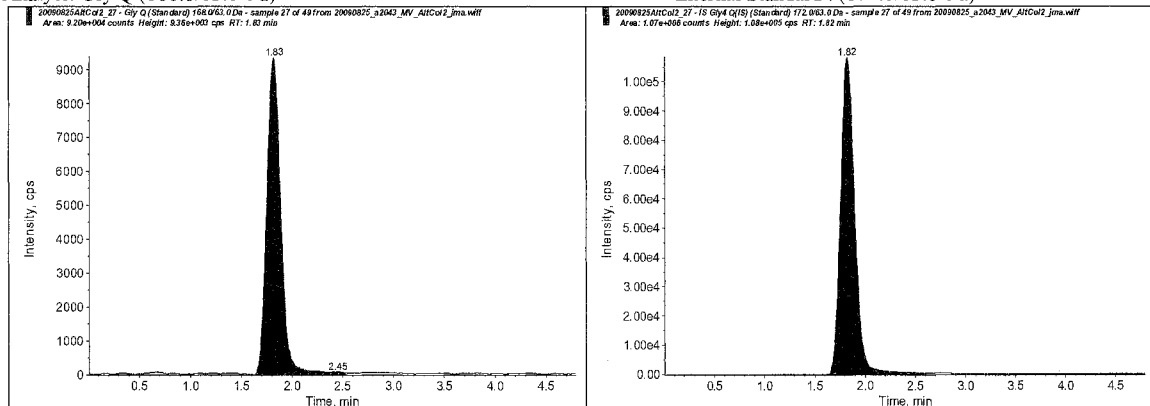
Sample No.: 27 Data Set: 20090825_a2043_MV_AltCoI2_jma.rdb

Analyte: Gly Q (168.0/63.0 Da)

RT: 1.83 min

PPM Found: 0.00476

Internal Standard : (172.0/63.0 Da)



Sample Id.: Corn Stover Control

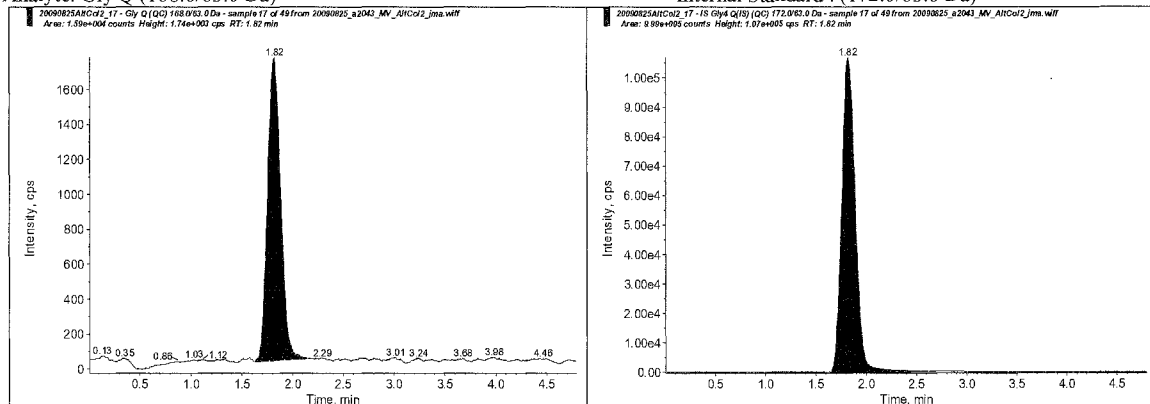
Sample No.: 17 Data Set: 20090825_a2043_MV_AltCoI2_jma.rdb

Analyte: Gly Q (168.0/63.0 Da)

RT: 1.82 min

PPM Found: 0.00803

Internal Standard : (172.0/63.0 Da)



Sample Id.: Corn Stover Fortified at 0.05 ppm Glyphosate

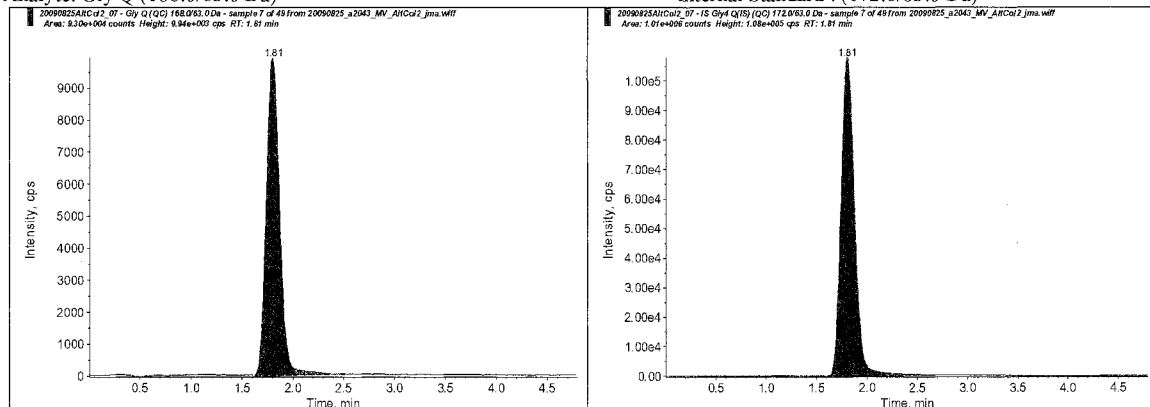
Sample No.: 07 Data Set: 20090825_a2043_MV_AltCoI2_jma.rdb

Analyte: Gly Q (168.0/63.0 Da)

RT: 1.81 min

PPM Found: 0.05110

Internal Standard : (172.0/63.0 Da)

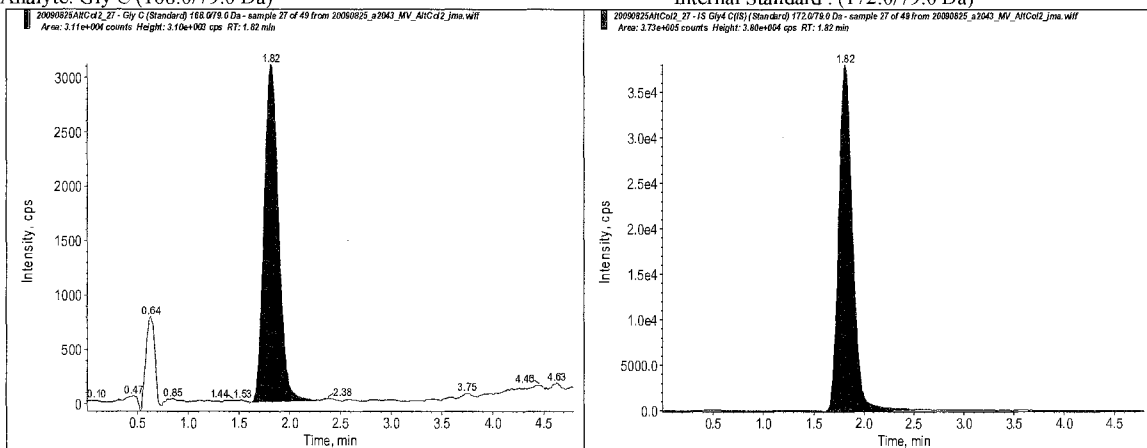


(HPLC Column: Bio-Rad 30 X 4.6 mm Cation H)

MRM Chromatograms for Glyphosate Confirmation Ion in Corn Stover

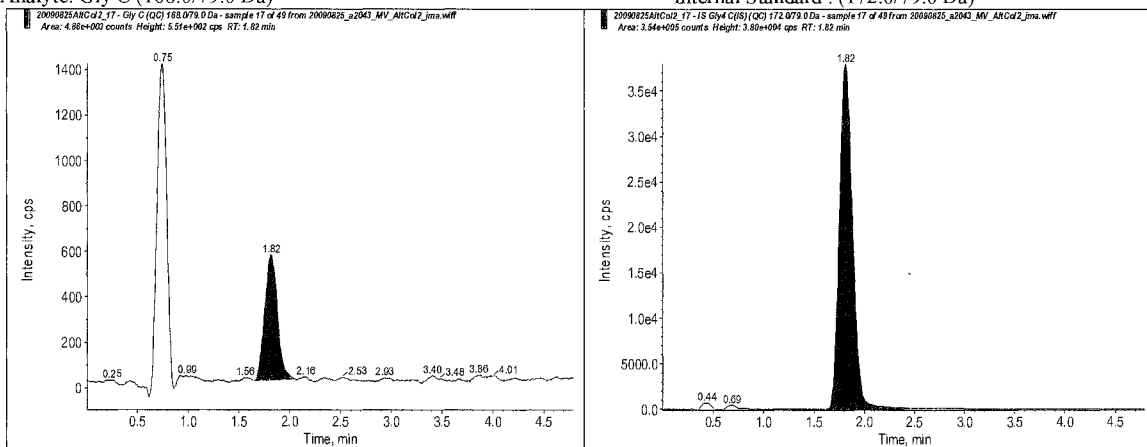
Sample Id.: Calibration Standard 0.005 µg/mL Glyphosate
Sample No.: 27 Data Set: 20090825_a2043_MV_AltCol2_jma.rdb
Analyte: Gly C (168.0/79.0 Da)

RT: 1.82min PPM Found: 0.00474
Internal Standard : (172.0/79.0 Da)



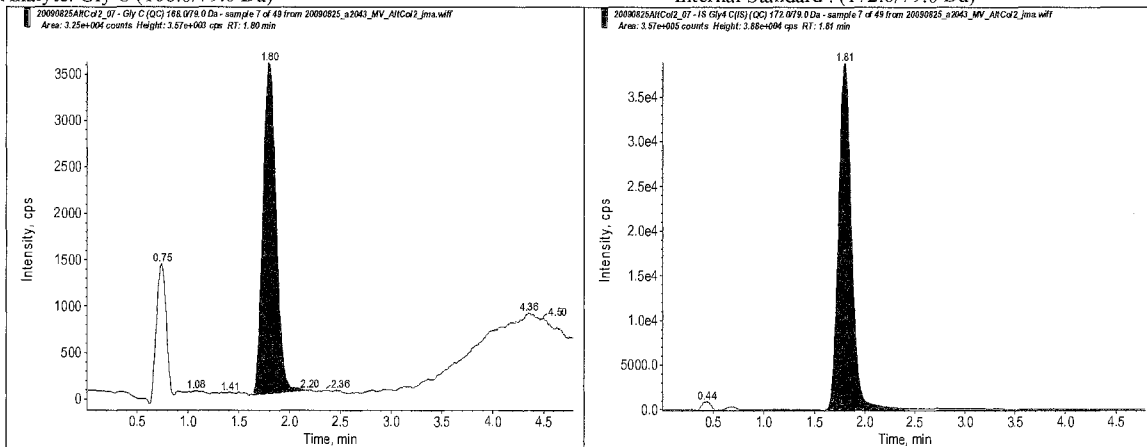
Sample Id.: Corn Stover Control
Sample No.: 17 Data Set: 20090825_a2043_MV_AltCol2_jma.rdb
Analyte: Gly C (168.0/79.0 Da)

RT: 1.82min PPM Found: 0.00796
Internal Standard : (172.0/79.0 Da)



Sample Id.: Corn Stover Fortified at 0.05 ppm Glyphosate
Sample No.: 07 Data Set: 20090825_a2043_MV_AltCol2_jma.rdb
Analyte: Gly C (168.0/79.0 Da)

RT: 1.80min PPM Found: 0.05190
Internal Standard : (172.0/79.0 Da)



(HPLC Column: Bio-Rad 30 X 4.6 mm Cation H)

MRM Chromatograms for AMPA Quantitation Ion in Corn Stover

Sample Id.: Calibration Standard 0.005 ug/mL AMPA

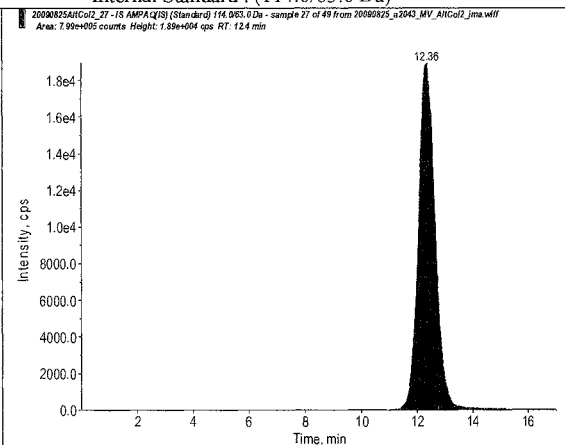
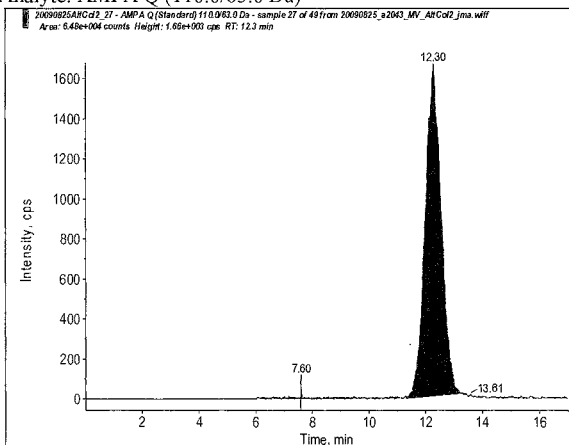
Sample No.: 27 Data Set: 20090825_a2043_MV_AltCol2_jma.rdb

Analyte: AMPA Q (110.0/63.0 Da)

RT: 12.3 min

PPM Found: 0.00488

Internal Standard : (114.0/63.0 Da)



Sample Id.: Corn Stover Control

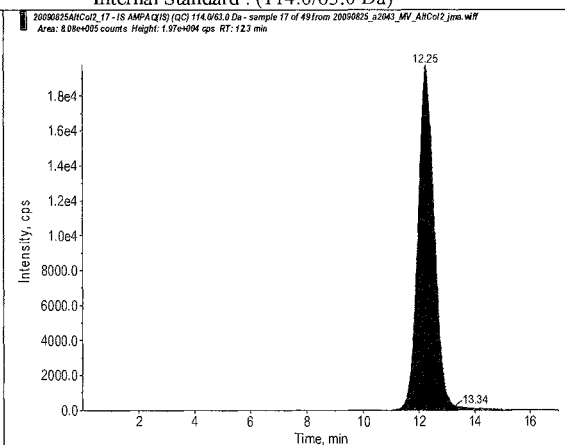
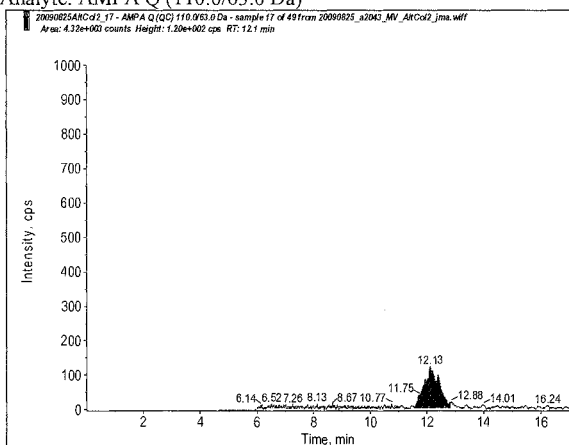
Sample No.: 17 Data Set: 20090825_a2043_MV_AltCol2_jma.rdb

Analyte: AMPA Q (110.0/63.0 Da)

RT: 12.1 min

PPM Found: 0.00434

Internal Standard : (114.0/63.0 Da)



Sample Id.: Corn Stover Fortified at 0.05 ppm AMPA

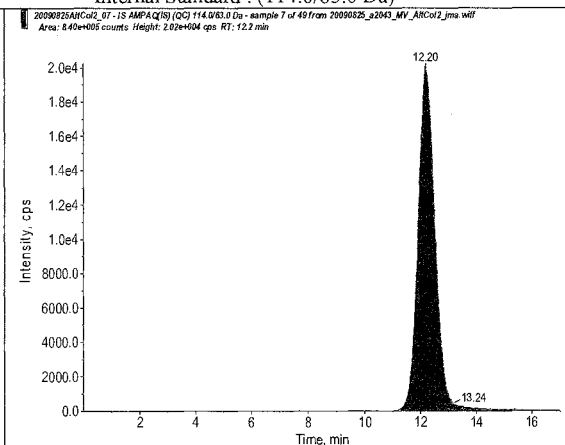
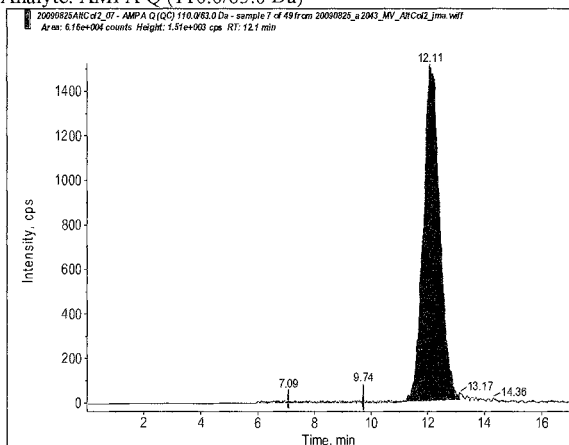
Sample No.: 07 Data Set: 20090825_a2043_MV_AltCol2_jma.rdb

Analyte: AMPA Q (110.0/63.0 Da)

RT: 12.1 min

PPM Found: 0.04430

Internal Standard : (114.0/63.0 Da)



(HPLC Column: Bio-Rad 30X4.6 mm Cation H)

MRM Chromatograms for AMPA Confirmation Ion in Corn Stover

Sample Id.: Calibration Standard 0.005 ug/mL AMPA

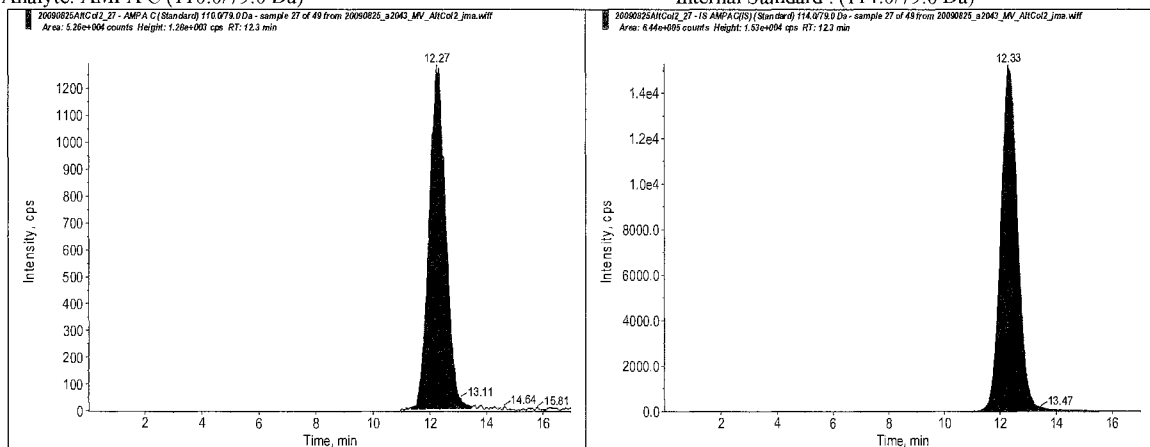
Sample No.: 27 Data Set: 20090825_a2043_MV_AltCoI2_jma.rdb

Analyte: AMPA C (110.0/79.0 Da)

RT: 12.3 min

PPM Found: 0.00477

Internal Standard : (114.0/79.0 Da)



Sample Id.: Corn Stover Control

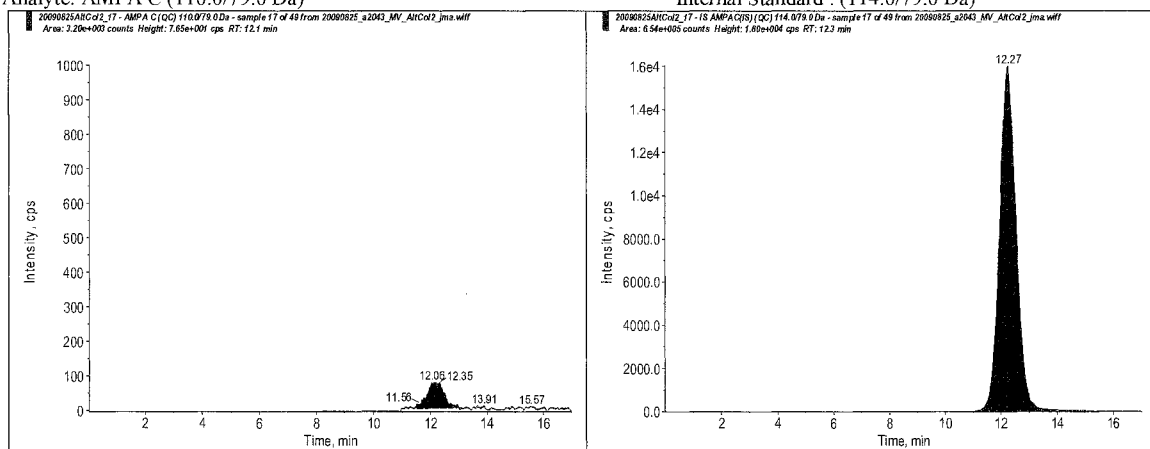
Sample No.: 17 Data Set: 20090825_a2043_MV_AltCoI2_jma.rdb

Analyte: AMPA C (110.0/79.0 Da)

RT: 12.1 min

PPM Found: 0.00359

Internal Standard : (114.0/79.0 Da)



Sample Id.: Corn Stover Fortified at 0.05 ppm AMPA

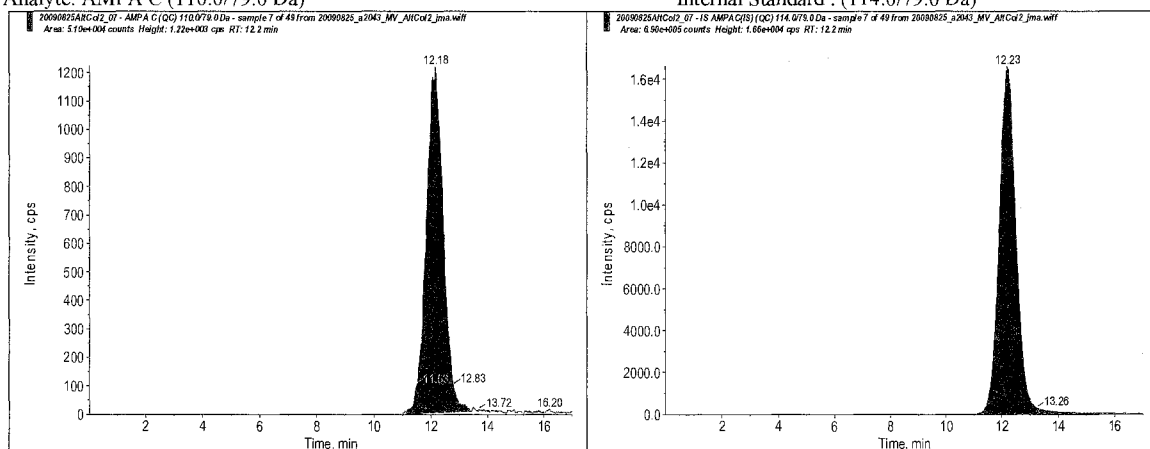
Sample No.: 07 Data Set: 20090825_a2043_MV_AltCoI2_jma.rdb

Analyte: AMPA C (110.0/79.0 Da)

RT: 12.2 min

PPM Found: 0.04330

Internal Standard : (114.0/79.0 Da)



(HPLC Column: Bio-Rad 30 X 4.6 mm Cation H)