

VOLUME ____ OF ____ OF SUBMISSION

S-METOLACHLOR: METHOD

TITLE

Analytical Method for the Enantioselective Determination of Residues of S-Metolachlor as Metabolites SYN-506357 and SYN-508500 in Agricultural Commodities by Chiral High Performance Liquid Chromatography with Mass Spectrometric Detection

DATA REQUIREMENT

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VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 67

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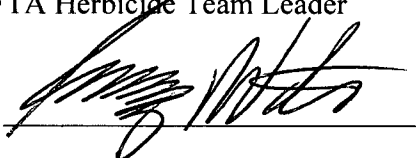
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Date: 6/26/03

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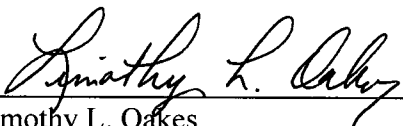
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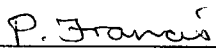
Since this volume contains an analytical method, and is not a final report of a GLP study, a separate Compliance Statement is not appropriate or necessary. Chromatograms shown in this method are for illustrative purposes only and are not representative of GLP data.



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1.0 SUMMARY AND INTRODUCTION

1.1 SCOPE

S-metolachlor (CGA-77102, S-2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide) is a selective herbicide developed for the control of a large number of grassy weeds in corn, soybeans, sorghum and a number of other major and minor crops. CGA-77102 consist predominately of the s-enantiomers. The S-diastereomers have been shown to exhibit increased biological efficacy toward control of problem weeds as compared to the R-diastereomers.

This method can be utilized to selectively determine residues of CGA-77102 in crops as a pair of acid hydrolysis products which maintain their enantiomeric configuration. This method utilizes a chiral high performance liquid chromatography separation with tandem mass selective detection (LC/MS/MS) as a highly selective technique to separate and quantify residue levels of the two chiral hydrolysis products SYN506357 (S-CGA-37913) and SYN508500 (S-CGA-49751).

The limit of detection (LOD), defined as the lowest amount of standard injected is 0.0625 ng and 0.25 ng for SYN506357 and SYN508500, respectively. The limit of quantitation (LOQ), defined as the lowest fortification level used for procedural recoveries, is 0.03 ppm and 0.05 ppm for SYN506357 and SYN508500, respectively. The chemical structures of CGA-24705 (racemic metolachlor), CGA-77102 (S-metolachlor) SYN506357 and SYN508500 are shown in Figure 1.

The representative substrates tested to date are corn grain, leaf lettuce, tomatoes, and potatoes fortified with SYN506357 and SYN508500.

For the purpose of consistency, the following terminology will be used to describe procedure and results presented in this method:

- The term (RS) racemate will be used to describe and reference substances containing metolachlor, which is a 50/50 racemic mixture of the (S) and (R) enantiomers. The hydrolysis products CGA-37913 and CGA-49751 also represent racemic mixtures.
- The term (S) enantiomer will be used to describe the predominately (S) enantiomer product CGA-77102 and the (S) enantiomers of the hydrolysis products. The (S) hydrolysis products are designated as SYN506357 (S-CGA-37913) and SYN508500 (S-CGA-49751).
- The (S) enantiomer is present at approximately 50% in the (RS) racemate and approximately 88% by weight in S-metolachlor. The hydrolysis

products derived from metolachlor and S-metolachlor maintain these same percentages.

1.2 METHOD HISTORY

The current tolerance enforcement method for s-metolachlor (CGA-77102) is AG-338¹. Analytical Method AG-612² (issued 7/93) is an updated version of AG-338. In these methods, s-metolachlor residues are converted to the hydrolysis products CGA-37913 and CGA-49751 by refluxing for 16 hours with 6N hydrochloric acid. In 2000, EN-CAS Analytical Laboratories issued ENC-5/99³ which updated AG-612, and changed the method of determination from gas chromatography with nitrogen/phosphorous detection to liquid chromatography with LC/MS/MS detection. The updated method ENC-5/99 uses the same sample extraction and clean-up procedures as AG-338 and AG-612. This method modifies ENC-5/99 to include chiral chromatography and LC/MS/MS determination, thus providing a highly selective technique to separate and quantify residue levels of the two chiral hydrolysis products SYN506357 (S-CGA-37913) and SYN508500 (S-CGA-49751).

1.3 PRINCIPLE

A sample is refluxed in 6N HCl for 16 ±1 hours. The cooled sample is filtered and residues of SYN506357 and SYN508500 are partitioned from a pH adjusted aliquot into dichloromethane (DCM). The sample is further purified using an alumina column then brought to appropriate final volume. Quantitation is achieved using a reverse phase chiral column and high pressure liquid chromatography with tandem mass spectrometer detection (LC/MS/MS). See Table 1 and 2 for specific details concerning the mass spectrometric detection of SYN506357 and SYN508500, respectively.

A flowchart of these analytical procedures is presented in Figure 2.

2.0 MATERIALS AND METHODS

2.1 APPARATUS/EQUIPMENT

NOTE: All apparatus listed may be replaced by equivalent apparatus from alternative sources if experimental verification supports such substitutions.

- 2.1.1 French square bottles with Teflon lined caps, 8-oz.
- 2.1.2 Graduated cylinders, 25-mL, 100-mL and 250-mL.
- 2.1.3 Volumetric pipettes, (various 1-25 mL sizes).
- 2.1.4 Separatory funnels, 500-mL with stoppers.
- 2.1.5 Erlenmeyer flasks, 250-mL or 500-mL, with 24/40 ground glass fittings.
- 2.1.6 Powder funnel, aluminum, 9-cm diameter with 1-cm diameter neck.
- 2.1.7 Chromatography column, 1.5-cm ID x 20-cm long with frit, Bio-Rad Cat # 737-1521.2.7
- 2.1.8 Column Reservoir, 250-mL, Bio-Rad Cat # 731-0003.
- 2.1.9 Teflon Valve Liners, Supelco Cat.# 5-7059.
- 2.1.10 Teflon Tubing 0.02” ID x 1/16” OD, Upchurch Scientific Cat.# 1548.
- 2.1.11 Glass Boiling beads, Fisher Cat.# 11-312B.
- 2.1.12 Heating Mantle for 500-mL flask.
- 2.1.13 Allihn, bulb-type water-cooled, condensers.
- 2.1.14 Fluted Filter Papers, 24-cm, Whatman 2V, Whatman Cat.# 120-2240.
- 2.1.15 Large glass funnels, ribbed.
- 2.1.16 Mettler analytical balance capable of five decimal accuracy, for weighing analytical standards.

2.1.17 Absorbent Sanitary Bulk Cotton, Fisher Cat.# 07-900.

2.1.18 Rotary Evaporator, Buchi or equivalent

2.2 REAGENTS

NOTE: All reagents listed may be replaced by equivalent reagents from alternative sources if experimental verification supports such substitutions.

2.2.1 Water, high purity, EM Science OMNI-SOLV
Cat.# EM-WX0004-1.

2.2.2 Water, deionized (DI).

2.2.3 Dichloromethane (DCM), pesticide grade, Fisher Cat.# D142-4.

2.2.4 Acetone, Optima grade, Fisher Cat.# A929-4.

2.2.5 Hydrochloric acid (HCl), 12N, Fisher Cat.# A144-212.

2.2.6 Sodium hydroxide solution (NaOH), 50% (w/w), Fisher
Cat.# SS254-4.

2.2.7 Methanol (MeOH), Optima grade, Fisher Cat.# A454-4.

2.2.8 Sodium sulfate anhydrous (granular) [Na₂SO₄], Mallinkrodt
Chemicals Cat.# 8024.

2.2.9 Alumina B, Activity I, ICN Biomedicals Cat.# 02072.

2.2.10 Deactivated Alumina, 30% (w/w) deactivated. [Add 30-mL of high purity water to 100-mL of Activity I Alumina in a glass bottle (>150mL capacity). Cap tightly and shake vigorously for several minutes to break-up all clumps. Allow to equilibrate >4 hours before use. Prepared alumina is highly deactivated and does not require storage in a dessicator.]

2.2.11 Sodium carbonate monohydrate (Na₂CO₃), certified A.C.S., ICN
Biomedicals Cat.# 152574.

2.2.12 Acetonitrile, HPLC grade, Burdick & Jackson Cat.# 015-4.

2.2.13 2-Propanol (isopropanol, Fisher Cat# A416SK-4.

2.2.14 6N HCl, Add 1000 mL of 12N HCl to 1000 mL of DI water.

- 2.2.15 25% NaOH, Add 500 mL of 50 % (w/w) NaOH to 500 mL DI water.
- 2.2.16 1M Na₂CO₃, Dissolve 247.96 g of Na₂CO₃ into 2.0 L of DI water.
- 2.2.17 5% water in acetone, Add 50 mL of high purity water to a 1000-mL volumetric flask and dilute to volume with acetone.
- 2.2.18 70:30 water:acetonitrile (ACN), combine 700 mL of high purity water with 300 mL ACN.
- 2.2.19 95/5 isopropanol/methanol, combine 950 mL of isopropanol with 50 mL of methanol for 95/5 IPA/MeOH.
- 2.2.20 75:25 water:95/5 IPA/MeOH, combine 750 mL of high purity water with 250 mL of 95/5 IPA/MeOH.

2.3 ANALYTICAL PROCEDURE

2.3.1 Extraction

2.3.1.1 Sample Preparation

Prepare crop substrates using procedures outlined in the Syngenta Product Safety Group SOP 7.21, Revision 3.

2.3.1.2 Extraction of Substrates

Weigh 25-g (± 0.1 g) of sample into a 500-mL round bottom flask (RBF). Fortify if appropriate for procedural recoveries at this point. To the other samples, add the same volume of acetone as in the fortified samples. Add 200-mL of 6 N HCl and 4-10 boiling beads. Attach a water-cooled allihn condenser to the RBF and place on heating mantle. Reflux the sample at a steady boil for 16 ± 1 hours.

Allow the sample to cool before removing it from the condenser. Place a Whatman[®] 2V filter paper into a large ribbed funnel sitting atop an 8-oz. French Square bottle (FSB). Crimp the bottom point of the paper cone to keep it from flattening out and rupturing during filtration. Once the sample is cooled to room temperature (this can be accelerated by using an ice bath), pour the entire sample through the filter without rinsing.

2.3.2 Partition

Measure a 25.0-mL aliquot of the sample and place it into a clean 500-mL erlenmeyer flask. Add 100 mL of DI water and chill the sample in an ice bath for 5 – 10 minutes in order to sufficiently cool the samples. Slowly add 12.5 mL of 25% (w/w) NaOH while stirring and then add 25 mL of 1M Na₂CO₃. Check the pH with a meter or with pH paper. It should be between 8.0 and 10.5, if not, add an additional 5 mL of 1M Na₂CO₃ until the desired pH is achieved.

Pour the sample into a 500-mL separatory funnel and add 50 mL of DCM (Due to DCM fumes all partitions and evaporating should be done with adequate ventilation). Stopper and shake the sample, with regular venting, for 20 - 30 seconds.

Allow the sample to sit, undisturbed, for ca. 10 minutes. [Note: In many samples a loose emulsion is formed in the lower, DCM, layer. Let the sample sit undisturbed. This let's the emulsion stiffen, allowing the majority of it to be easily broken-up]. Use a stirring rod or pipette to stir the sample and break-up the majority of any emulsion. Drain the DCM phase (lower layer) through a cotton plug in the neck of a powder funnel into a FSB, stopping at any emulsion layer.

Repeat the partition one more time with 50 mL of DCM, combining the DCM aliquots. Allow the clarified portion of the sample to pass through the cotton plug before draining the majority of any emulsion into the funnel. Leave the last 1 – 2 mL of any emulsion behind in the separatory funnel. As soon as the DCM stops dripping through the cotton plug, remove the funnel to prevent any of the aqueous in the emulsion from dripping into the sample.

2.3.3 Alumina Column Cleanup

Prepare an alumina column by attaching a 250-mL reservoir to the top of the column and a flow restrictor to the bottom (Make a restrictor by inserting a 3-cm piece of 0.02" ID tubing inside the Valve Liner tubing, alternatively use a Teflon stopcock). Add 5 mL of 30% deactivated alumina (measured in a graduated tube, ca. 6 grams) to a 1.5-cm diameter chromatography column. Add ca. 1.5-cm of Na₂SO₄ to the top of a level bed of alumina and precondition the column with 10 – 15 mL of DCM (to waste).

Place a clean 250-mL or 500-mL Erlenmeyer flask under the column and add the sample to the column (collect). Maintain a flowrate up to ca. 5 mL/minute. Once the sample has passed through, add 10-mL of DCM to the column and combine the DCM portions (contains SYN506357). Proceed to step 4.

Place a clean 250-mL or 500-mL flask under the alumina column and add 75 mL of 5% water in acetone to the column reservoir (collect, contains SYN508500). Proceed to Step 5.

2.3.4 Rotary Evaporation and Final Volume (SYN506357)

Place the sample on a rotary evaporator at $\leq 25^{\circ}\text{C}$. Evaporate the sample to dryness. Make any necessary dilutions with approximately 70/30 water:ACN (this ratio will be determined based on the optimization of the water/ACN ratio necessary to get maximum chiral separation) dispense the sample into a vial suitable for LC/MS/MS analysis. It is recommended that a final volume of 10-mL or greater be used to reduce ion suppression on the mass spectrometer.

2.3.5 Rotary Evaporation and Final Volume (SYN508500)

Place the sample on a rotary evaporator at 35 - 40°C. Reduce the volume of the sample to ca. 3 mL. When the solvent stops condensing and draining into the rotary evaporator catch flask, this generally means the sample is almost entirely water.

(Note: The OD-RH chiral column is very sensitive to acetone, so be extremely careful to ensure that all acetone has been removed from the samples. The use of an N-evap can assist in assuring that only aqueous sample remains).

Transfer the contents of the Erlenmeyer flask to a graduated centrifuge tube using a pipette. If the volume is greater than 4.0 mL then place the sample on a nitrogen evaporator apparatus and reduce the volume to 3.0 mL \pm 0.1 mL. (It is extremely important that all acetone has been removed from the sample).

Rinse the flask with 3.0 ml of water and add to the graduated tube (total of 6 mL water). Dilute the sample with 2.0 mL of 95/5 2-propanol/methanol to give a final volume of 8 ml, and dispense the sample into a vial suitable for LC/MS/MS analysis. (If further dilution is required, add an appropriate volume of 75/25 (water/(95/5) 2-propanol/methanol).

2.4 INSTRUMENTATION

2.4.1 Description of Operating Conditions

Analyze samples using reverse-phase chiral HPLC with tandem mass spectroscopic detection (LC/MS/MS). See Tables 1 and 2 for description of the system components and chromatographic and mass spectroscopic conditions.

2.4.2 Standardization

Standardize the LC/MS/MS system by injecting 25- μ L aliquots of standard solutions containing the analytes in the range of 0.005ng to 0.05ng for SYN506357 and 0.01ng to 0.05ng for SYN508500. Generate a linear regression from the data by comparing detector response and ng injected. See Figures 13-16 for examples of standard chromatograms and example calibration curves.

2.5 INTERFERENCES

The use of mass spectrometric detection (LC/MS/MS), provides a highly selective and specific technique for the analysis of SYN506357 and SYN508500. No interferences were encountered during the development of this method.

2.6 CONFIRMATORY TECHNIQUE

Due to the highly specific nature of mass spectroscopic detection, especially LC/MS/MS, no confirmatory techniques are required.

2.7 TIME REQUIRED

The extraction for this method consist of a 16 hour acid reflux. The cleanup portion for a set of six samples may be completed within a period of approximately 4-6 hours. A complete LC/MS/MS sequence consisting of six samples and six calibration standards may take an additional 6 hours when using an automated injection system.

2.8 MODIFICATIONS AND POTENTIAL PROBLEMS

None.

2.9 PREPARATION OF STANDARD SOLUTIONS AND SAMPLE FORTIFICATION PROCEDURES

2.9.1 Preparation of Stock Solutions

2.9.1.1 CGA-49751 Stock Solution - Using an analytical balance, accurately weigh 10 mg CGA-49751 into a

100-mL volumetric flasks. Dilute to the mark with acetone. The concentration of this stock is 100 ng/ μ L. Store in the freezer and in the dark. Stock solutions expire 6 months after preparation.

2.9.1.2 CGA-37913 Stock Solution - Using an analytical balance, accurately weigh 10 mg CGA-37913 into a 100-mL volumetric flasks. Dilute to the mark with acetone. The concentration of this stock is 100 ng/ μ L. Store in the freezer and in the dark. Stock solutions expire 6 months after preparation.

2.9.1.3 SYN506357 Stock Solution - Using an analytical balance, accurately weigh 10 mg SYN506357 into a 100-mL volumetric flasks. Dilute to the mark with acetone. The concentration of this stock is 100 ng/ μ L. Store in the freezer and in the dark. Stock solutions expire 6 months after preparation

2.9.1.4 SYN508500 Stock Solution - Using an analytical balance, accurately weigh 10 mg SYN508500 into a 100-mL volumetric flasks. Dilute to the mark with acetone. The concentration of this stock is 100 ng/ μ L. Store in the freezer and in the dark. Stock solutions expire 6 months after preparation

2.9.2 Fortification Standards

Standards may be made individually or mixed as appropriate for the study. An example of the preparation of the LOQ mixed fortification is shown below. Prepare a separate 10 ng/ μ L standard solution of SYN506357 and SYN508500 by adding 10.0 mL of the stock solution, prepared in previous section, to a 100 mL volumetric flask and dilute to the mark with acetone.

To prepare a mixed standard solution for the LOQ fortification of 0.03 ppm for SYN506357 and 0.05 ppm for SYN508500, add 7.5 mL of the 10.0 ng/ μ L SYN506357 standard solution and 12.5 ml of the 10.0 ng/ μ L SYN508500 standard solution to a 100 mL volumetric flask and dilute to the mark with acetone. The resulting standard is a mixed 0.75/1.25 ng/ μ L SYN506357/SYN508500 standard solution.

Prepare other fortification standard solutions as necessary. Working standards should be stored refrigerated when not in use.

2.9.3 Analytical Standard

SYN506357 - Prepare a 10.0 ng/μL standard solution by adding 10.0 mL of the stock solutions to a 100-mL volumetric flask. Evaporated the sample to complete dryness to remove all traces of acetone. Then bring standard up to volume with 70/30 water/acetonitrile. Make serial dilutions as shown in the following table. Working standards should be refrigerated when no in use.

SYN508500 - Prepare a 10.0 ng/μL standard solution by adding 10.0 mL of the stock solutions to a 100-mL volumetric flask. Evaporated the sample to complete dryness to remove all traces of acetone. Then bring standard up to volume with 75/25 water/(95/5) (2-propanol/methanol). Make serial dilutions as shown in the following table. Working standards should be refrigerated when no in use.

Standard to be added (ng/μL)	mL of standard	Final volume (mL)	Standard Concentration (ng/μL)
1.0	20	100	0.2
1.0	10	100	0.1
1.0	5	100	0.05
1.0	3	100	0.03
1.0	2	100	0.02
1.0	1	100	0.01
0.05	10	100	0.005

2.9.4 Sample Fortification Procedures

This method was designed for each set to include an untreated control sample and one or more control samples fortified immediately prior to sample reflux.

- 2.9.4.1 Add a known amount of the fortification standard to a twenty five-gram sample of control substrate prior to extraction.

An example fortification for the LOQ fortification is as follows:

Add 1.0 mL of combined 0.75/1.25 ng/ μ L fortification standard previously prepared. (These fortifications correspond to the 0.03 ppm/ 0.05 ppm LOQ fortifications established for this method for SYN506357 and SYN508500, respectively).

- 2.9.4.2 Proceed to step II.C.2.0 Typical recovery data for control and fortified control samples are given in Table IV. Calculations used to determine analyte recoveries are shown in Section II.J.2.

2.10 METHODS OF CALCULATION

2.10.1 Determination of Sample Residues

Inject 25- μ L aliquots of the final fractions prepared in section II.C.4 and II.C.5 into the LC/MS/MS system under the same conditions as the standards. Develop a calibration curve on the LC/MS/MS data system that plots the peak height of the abundance of the [m/z] positive ion against the amount of S-enantiomer reference material injected to determine the nanograms of S-enantiomer in the injected sample extract. Refer to the following criteria to determine which peaks within the chromatogram are the S-enantiomer. Chromatograms should closely resemble those shown in Figures 3 and 4 for SYN506357 and SYN508500, respectively.

SYN506357: Figure 3 shows a comparison between the racemic mixture of CGA-37913 and the S-enantiomer SYN506357. CGA-37913 is a doublet consisting of a 50/50 ratio of the R to S enantiomers. The SYN506357 chromatogram contains only the S enantiomer. The commercial formulation of s-metolachlor will contain an approximately 88/12 ratio between the active S enantiomer and

the inactive R enantiomer. Calibration will be based on the peak heights of the S-enantiomer.

SYN508500: Figure 4 shows a comparison of the racemic mixture CGA-49751 and S-enantiomer SYN508500. CGA-49751 consist of 8 enantiomers in a 50/50 ratio of the R to S enantiomers. Figure 4 demonstrates that not all eight of the isomers can be separated. However a distinct S and R peak can be baseline resolved. Calibration will be based on the peak height of the resolved S-enantiomer. The peak ratios of the resolved peaks have been shown to remain constant through a linear range.

To calculate the residue results, the mg injected must first be calculated as follows:

$$(1) \text{ mg injected} = \frac{(G)(V_a)(V_i)}{(V_e + W (M/100)) (V_f)}$$

G = amount of sample extracted (mg)

V_a = aliquot volume (mL)

V_e = extraction volume (mL)

V_i = injection volume (mL)

V_f = total volume of final injection solution (μL)

M = % moisture of sample

W = weight of sample (g)

To determine the corrected ppm of each analyte in the samples use equation 2:

$$(2) \text{ ppm} = \frac{(\text{ng analyte found}) \times 100 (F)}{(\text{mg sample injected})}$$

R% = recovery ratio given by equation (4)

F = conversion factor for the analyte to s-metolachlor equivalents = MW s-metolachlor / MW for analyte:

for SYN506357 = 1.47

for SYN508500 = 1.14

2.10.2 Determination of Procedural Recoveries

2.10.2.1 Determine the ng of the S enantiomer in the aliquot of the sample in the samples injected, and calculate the mg injected using the procedures describe in Section II.J.1.0. Calculate the final ppm values on the control

and fortified samples according to the following equation:

$$(3) \text{ ppm} = \frac{\text{ng analyte found}}{\text{mg sample injected}}$$

Determine the recovery factor by first subtracting the background detector response, if any, in the control sample from the analyte response in the recovery sample. Calculate the recovery factor as a percentage (R%) by the equation:

$$(4) \text{ R\%} = \frac{\text{ppm analyte found} - \text{ppm control}}{\text{ppm analyte added}} \times 100$$

Note: Analysis of controls, correction of recovery results for small control values and the use of the recovery factor is not required for tolerance enforcement purposes. They are used for method validation purposes. Sample residues will not be corrected for procedural recoveries and do not contain any molecular weight conversion factor as used in sample conversions.

3.0 RESULTS AND DISCUSSION

The extraction and work-up procedures were validated under Study No. 133-92 as Analytical Method AG-338 with modifications, and used for the analysis of control, fortified control and 14C-metolachlor treated corn and potato samples. Method AG-338 was later reissued as Analytical Method AG-612.

4.0 CONCLUSION

Syngenta Analytical Method 1848-01 is demonstrated to be a valid method for the selective determination of residues of CGA-77102 in crops as a pair of acid hydrolysis products SYN506357 and SYN508500. These products have been proven to maintain their enantiomeric configuration throughout the procedures of this analytical methodology.

Through the use of a chiral high performance liquid chromatography separation with tandem mass selective detection (LC/MS/MS), this method provides a highly selective technique to separate and quantify residue levels of the two chiral hydrolysis products SYN506357 (S-CGA-37913) and SYN508500 (S-CGA-49751). The percent enantiomeric excess (EE%) parameter allows a reliable and unbiased determination to be made as to whether a crop was treated with a formulation containing either metolachlor or s-metolachlor as its active ingredient.

5.0 TABLES AND FIGURES

TABLE 1. OPERATING PARAMETERS FOR THE DETERMINATION OF SYN506357 BY REVERSE PHASE LC/MS/MS

Sample Introduction: Waters' Alliance Model 2690 High Performance Liquid Chromatograph (HPLC) system, with autosampler, quaternary solvent gradient elution pump.

HPLC Column: Chiralcel OD-RH, (150 mm X 4.6 mm I.D.) (Chiral Technologies Inc. Part No. 14724)

HPLC Conditions:

Inj. Volume: 25 µL

Column Temp.: 25°C (Ambient)

Column Flowrate: 0.5 ml/min (max pressure = 1400 psi)

The HPLC system has a post-column "splitter" installed between the column exit and the detector, which directs ~0.375 mL/min of the column flow to waste and ~0.125 mL/min to the MS detector (approximately 4:1 split ratio).

Mobile Phase: The mobile phase needs to be fine tuned dependant of the analytical column. This can be done by injecting the racemate (CGA-37913) and adjusting the mobile phase (70/30 water:ACN) until desired separation is achieved. It is suggested that a standard of the racemate be injected with every analytical set to insure that adequate separation of the isomers is maintained.

MS Detector: Micromass Quattro LC™ Mass Spectrometer with electrospray ionization (EI) interface. Data processed using Masslynx™ software (Rev. 3.1 Build 4).

The MS detector must be tuned to achieve maximum sensitivity. This can be accomplished by infusing of a standard solution and maximizing detector response.

Ions:

Compound	Ion Mode	Masses Monitored (Q1/Q3)	Retention Time (min)
SYN506357	Positive	194.3/136.3 amu	26

Data Acquisition: The LC/MS/MS Masslynx software has the capability to calculate the parts per million of analyte in a crop substrate if the milligram amount of crop-equivalent injected is supplied to the instrument. However it is typically easier for an analyst to obtain the peak height data from the LC/MS/MS system and transcribe this data into the Multichrom Worksheet program (version ws.pas 1.3.1) to completely calculate parts per million of analyte in the test substrate.

TABLE 2. OPERATING PARAMETERS FOR THE DETERMINATION OF SYN508500 BY REVERSE PHASE LC/MS/MS

Sample Introduction: Waters' Alliance Model 2690 High Performance Liquid Chromatograph (HPLC) system, with autosampler, quaternary solvent gradient elution pump.

HPLC Column: Chiralcel OD-RH, (150 mm X 4.6 mm I.D.) (Chiral Technologies Inc. Part No. 14724)

HPLC Conditions:

Inj. Volume: 25 µL

Column Temp.: 35°C

Column Flowrate: 0.4 ml/min (max pressure = 1400 psi)

The HPLC system has a post-column "splitter" installed between the column exit and the detector, which directs ~0.300 mL/min of the column flow to waste and ~0.100 mL/min to the MS detector (approximately 4:1 split ratio).

Mobile Phase: The mobile phase needs to be fine tuned dependant of the analytical column. This can be done by injecting the racemate (CGA-49751) and adjusting the mobile phase 75/25 (water/(95/5) 2-propanol/methanol) until desired separation is achieved. It is suggested that a standard of the racemate be injected with every analytical set to insure that adequate separation of the isomers is maintained.

MS Detector: Micromass Quattro LC™ Mass Spectrometer with electrospray ionization (EI) interface. Data processed using Masslynx™ software (Rev. 3.1 Build 4).

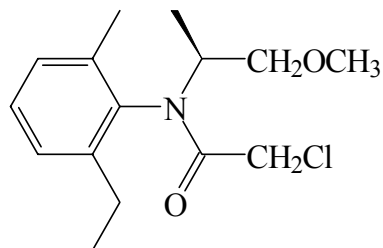
The MS detector must be tuned to achieve maximum sensitivity. This can be accomplished by infusing of a standard solution and maximizing detector response.

Ions:

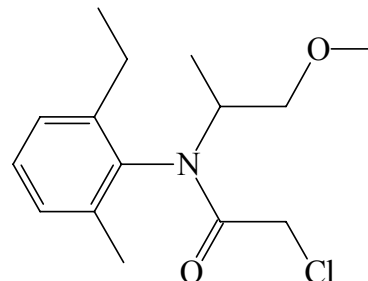
Compound	Ion Mode	Masses Monitored (Q1/Q3)	Retention Time (min)
SYN508500	Positive	249.9/203.8 amu	26

Data Acquisition: The LC/MS/MS Masslynx software has the capability to calculate the parts per million of analyte in a crop substrate if the milligram amount of crop-equivalent injected is supplied to the instrument. However it is typically easier for an analyst to obtain the peak height data from the LC/MSMS system and transcribe this data into the Multichrom Worksheet program (version ws.pas 1.3.1) to completely calculate parts per million of analyte in the test substrate.

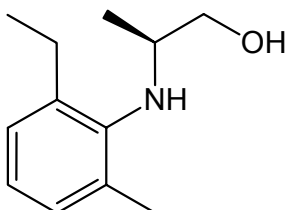
FIGURE 1. REFERENCE STANDARDS IDENTIFICATIONS AND STRUCTURES



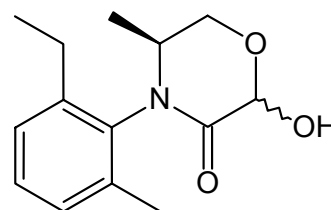
S-metolachlor (CGA-77102)
(S)-2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide



Metolachlor (CGA-24705)
2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide



SYN506357
(S) 2-[(2-ethyl-6-methylphenyl)amino]-1-propanol



SYN508500
(S) 6-hydroxy-3-methyl-4-(6'-ethyl-2'-methylphenyl)-oxomorpholine

NOTE: Store the reference materials in a refrigerator at a temperature of 0 - 5°C.

The figures shown above for SYN506357 and SYN508500 are the “S” configurations of the hydrolysis products CGA-37913 and CGA-49751, respectively.

FIGURE 2. METHOD FLOW CHART

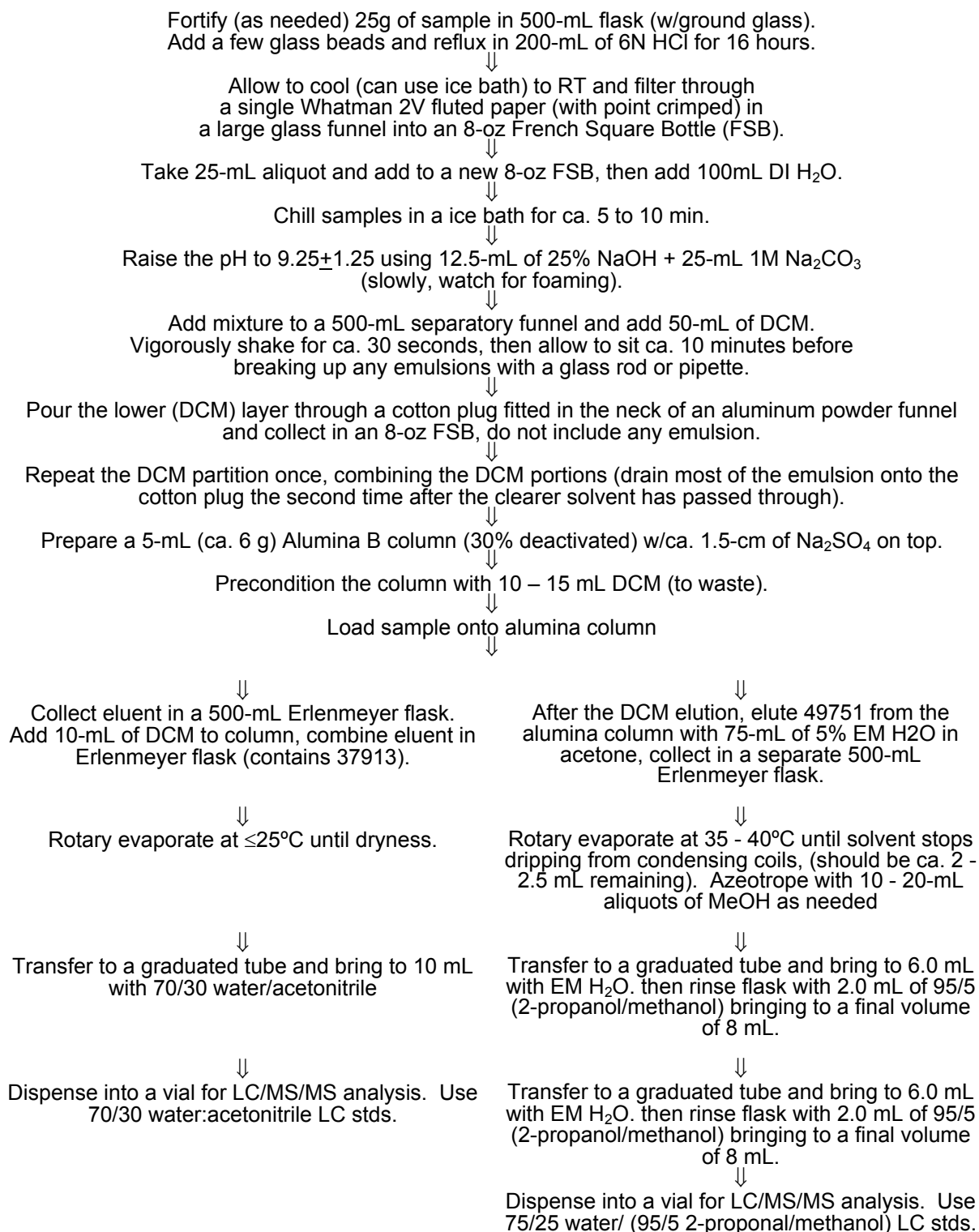
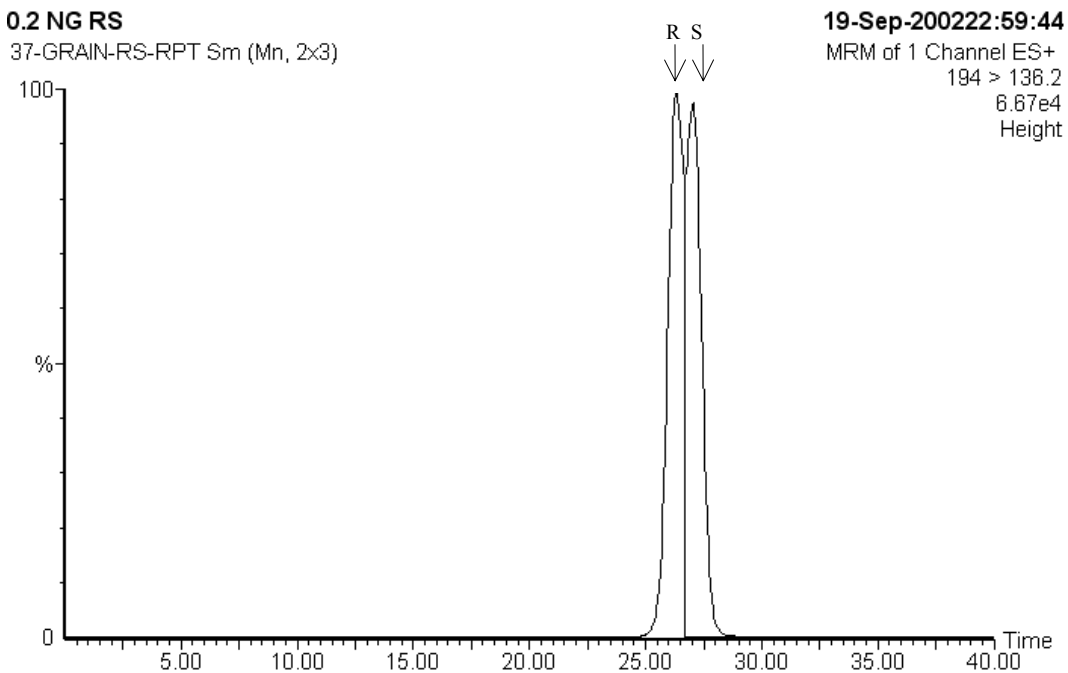
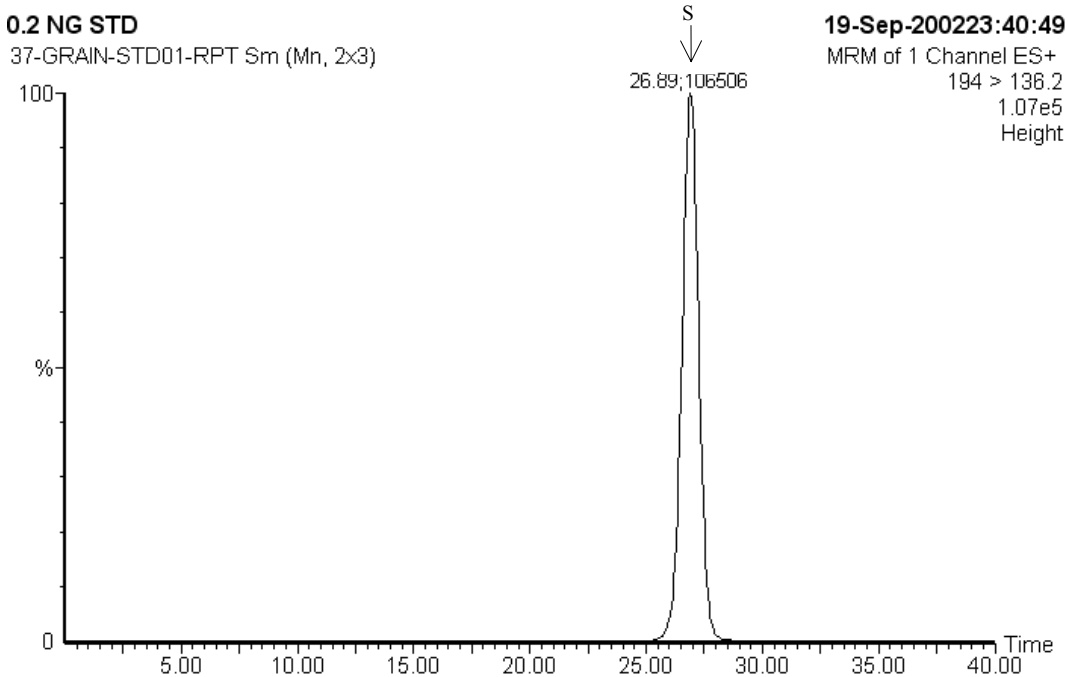


FIGURE 3. CGA-37913 AND SYN506357 STANDARD COMPARISONS

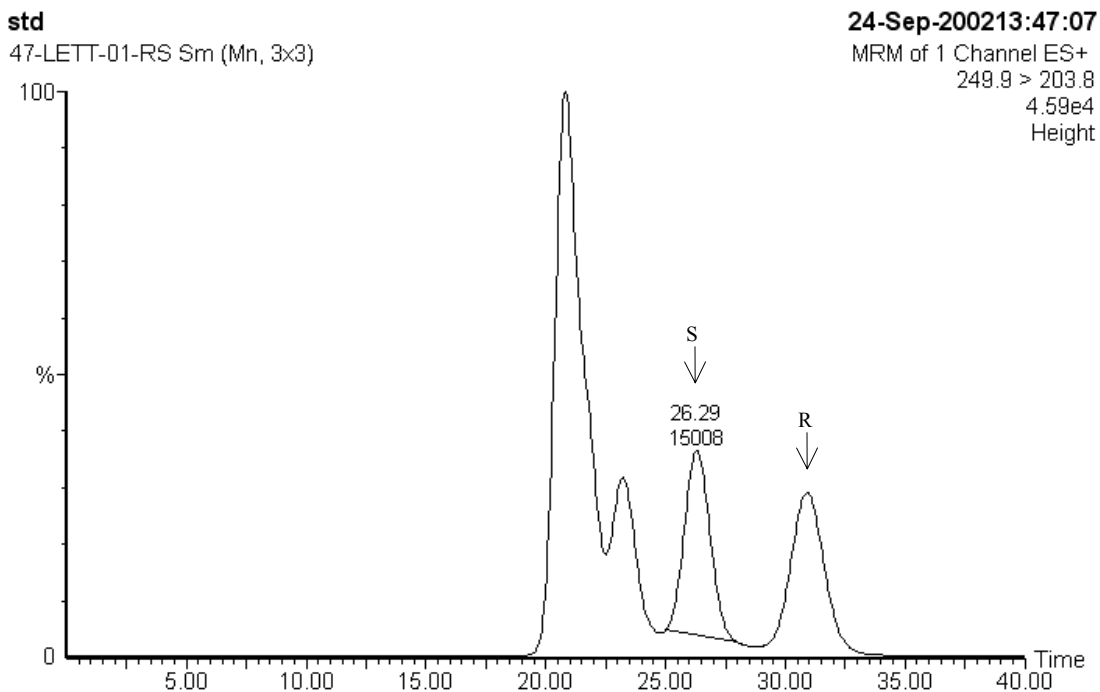


1. 5.0 ng standard, CGA-37913 (Racemic Mixture)

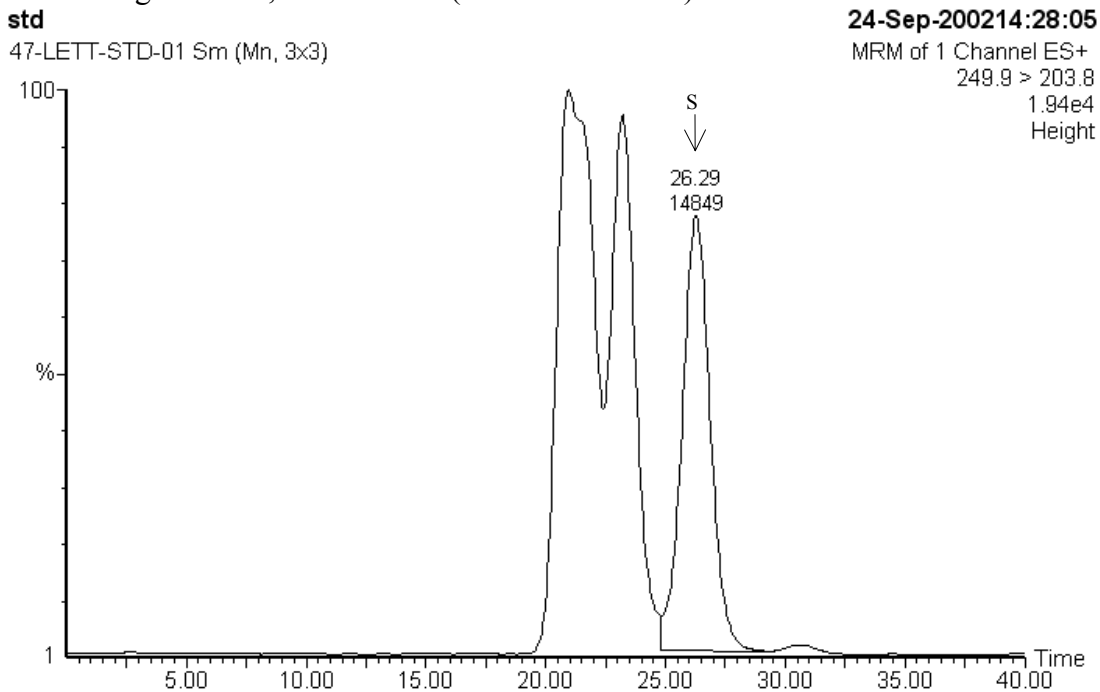


2. 5.0 ng standard, SYN506357 (S-CGA-37913)

FIGURE 4. CGA-49751 AND SYN508500 STANDARD COMPARISONS

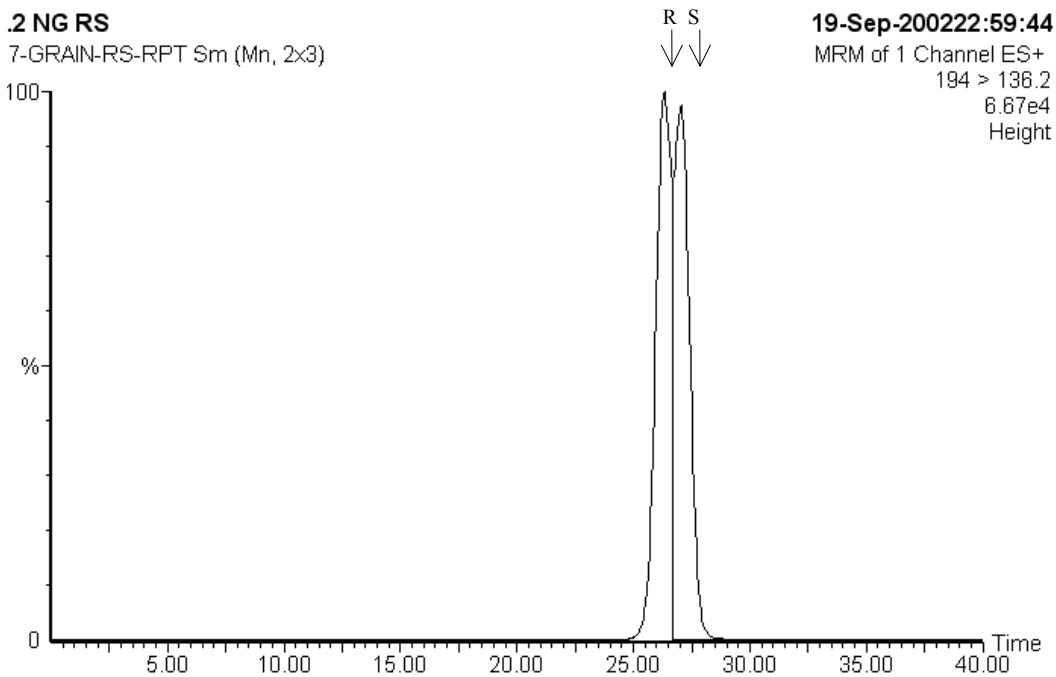


1. 1.25 ng standard, CGA-49751 (Racemic Mixture)

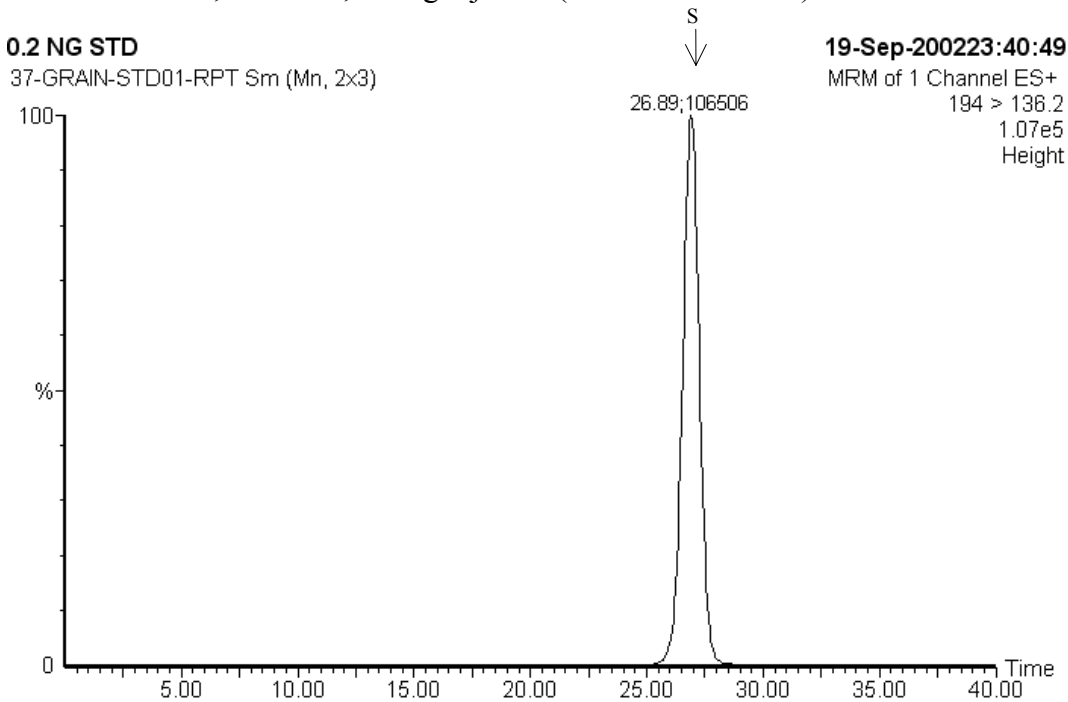


2. 1.25 ng standard, SYN508500 (S-CGA-49751)

FIGURE 5. CHROMATOGRAMS SYN506357 IN CORN GRAIN

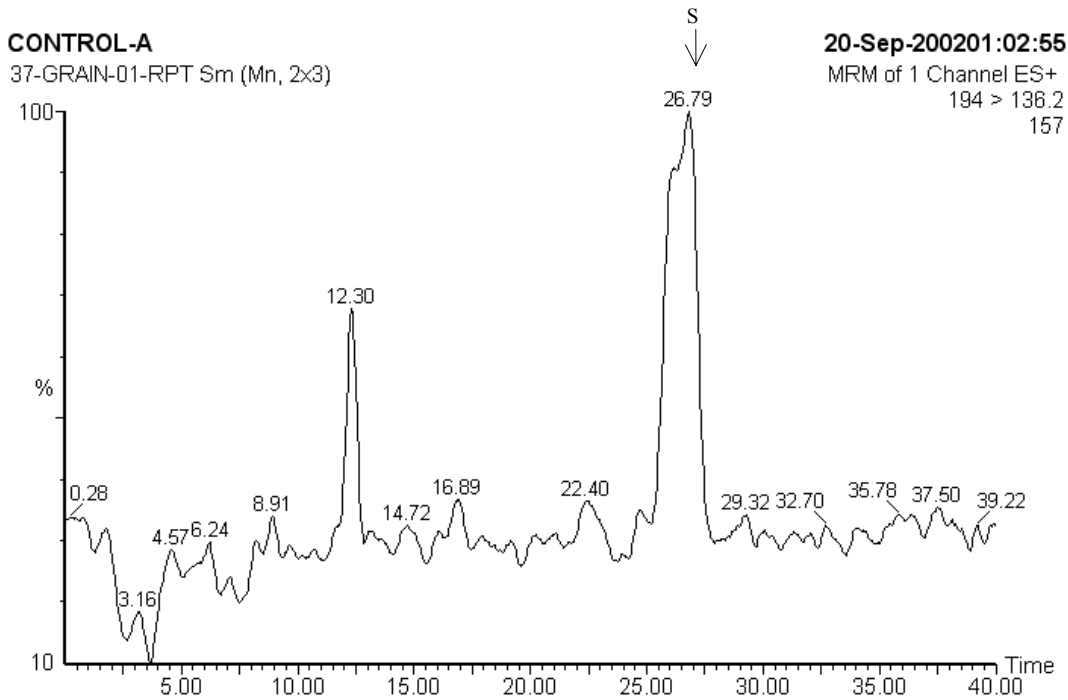


1. CGA-37913, Standard, 5.0 ng injected (Racemic Standard)

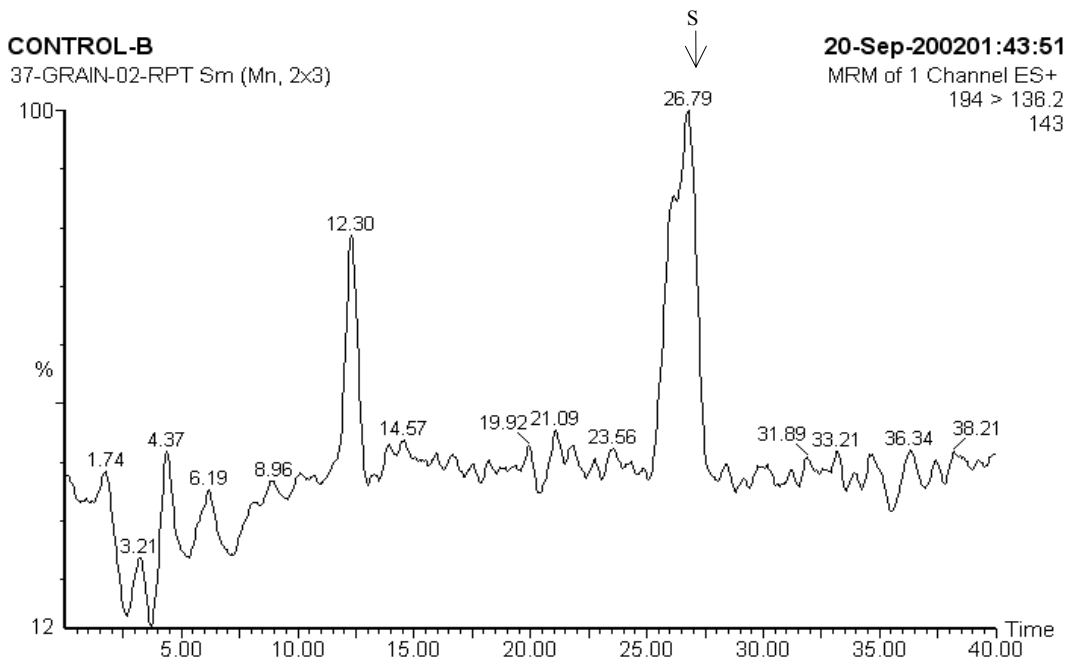


2. SYN506357 Standard, 5.0 ng injected

FIGURE 5. CHROMATOGRAMS SYN506357 IN CORN GRAIN (CONT.)

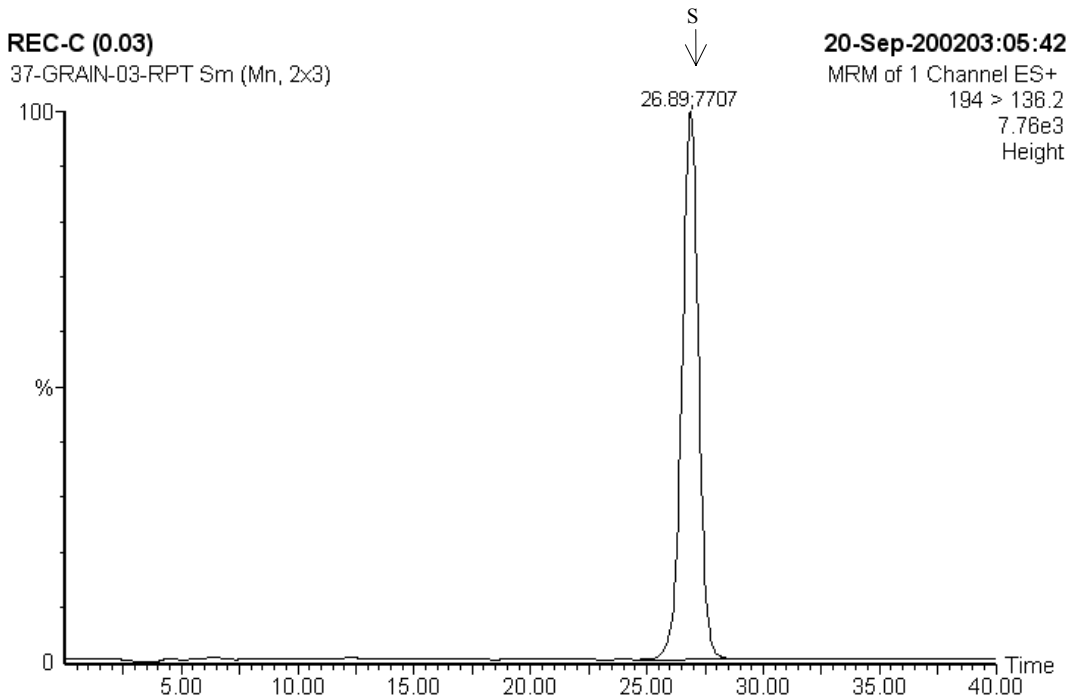


3. control, 15.4 mg injected, 0.00 ng found, <0.03 ppm

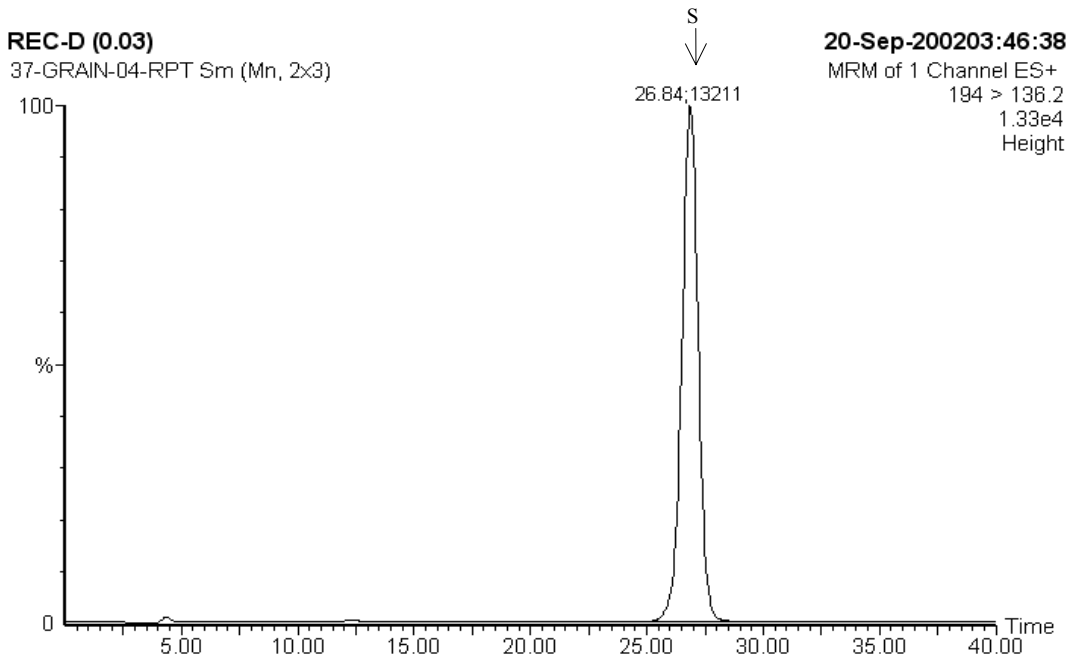


4. control, 15.4 mg injected, 0.00 ng found, <0.03 ppm

FIGURE 5. CHROMATOGRAMS SYN506357 IN CORN GRAIN (CONT.)

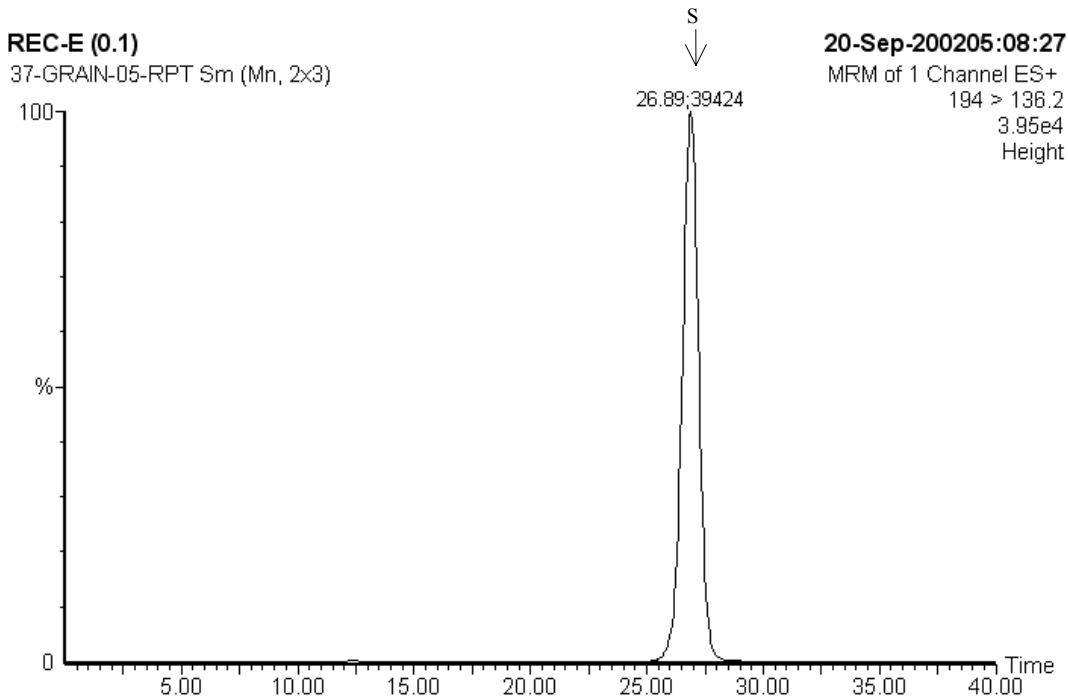


5. control + 0.03 ppm SYN506357, 15.4 mg injected, 0.381 ng found, 0.025 ppm, 82.6% recovery

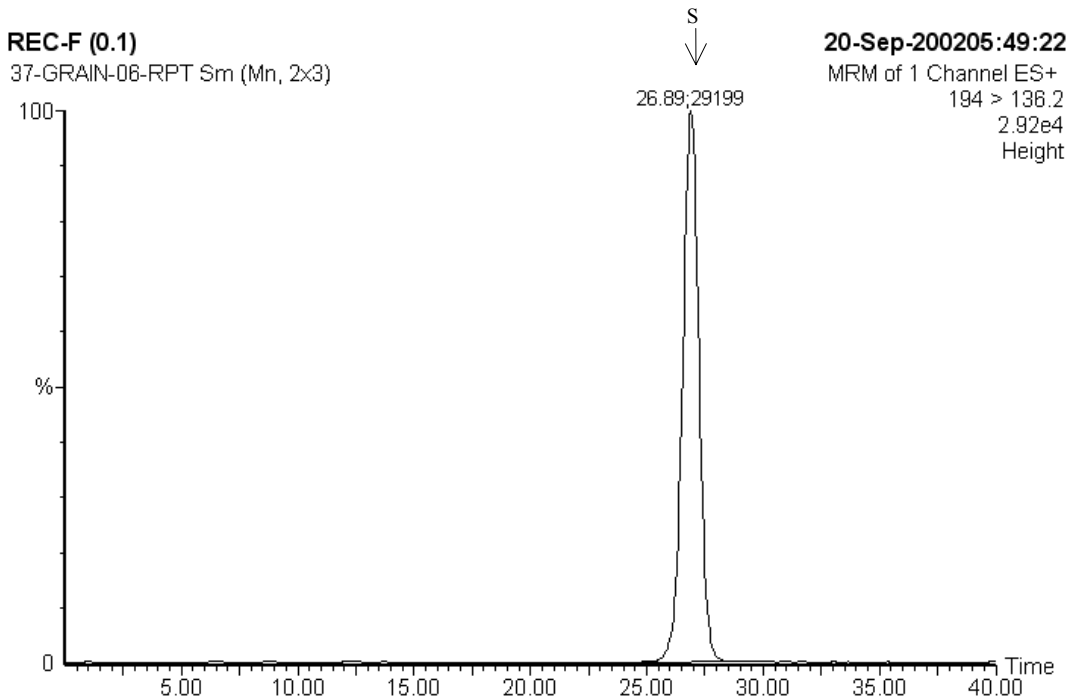


6. control + 0.03 ppm SYN506357, 15.4 mg injected, 0.644 ng found, 0.042 ppm, 140% recovery

FIGURE 5. CHROMATOGRAMS SYN506357 IN CORN GRAIN (CONT.)

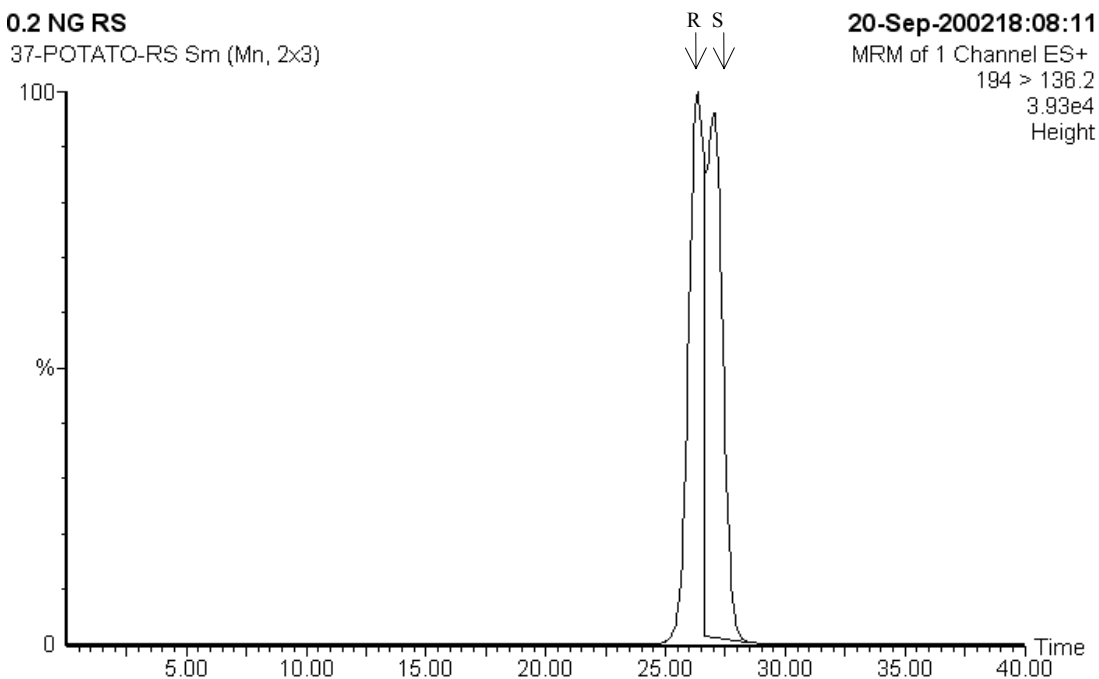


7. control + 0.10 ppm SYN506357, 15.4 mg injected, 1.90 ng found, 0.123 ppm, 123% recovery

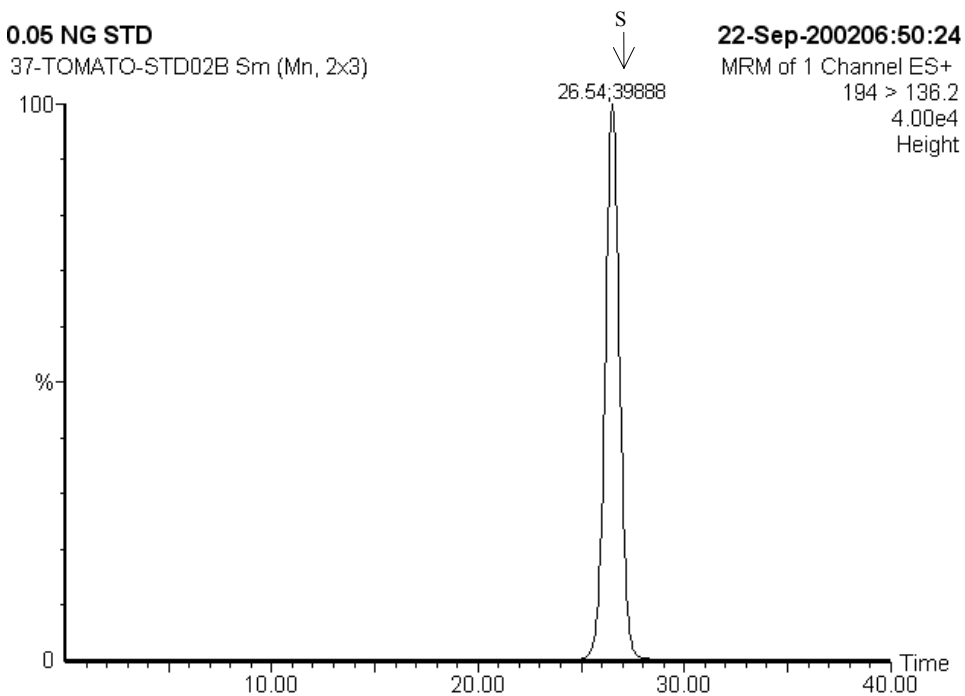


8. control + 0.10 ppm SYN506357, 15.4 mg injected, 1.41 ng found, 0.091 ppm, 91.4% recovery

FIGURE 6. CHROMATOGRAMS SYN506357 IN TOMATO

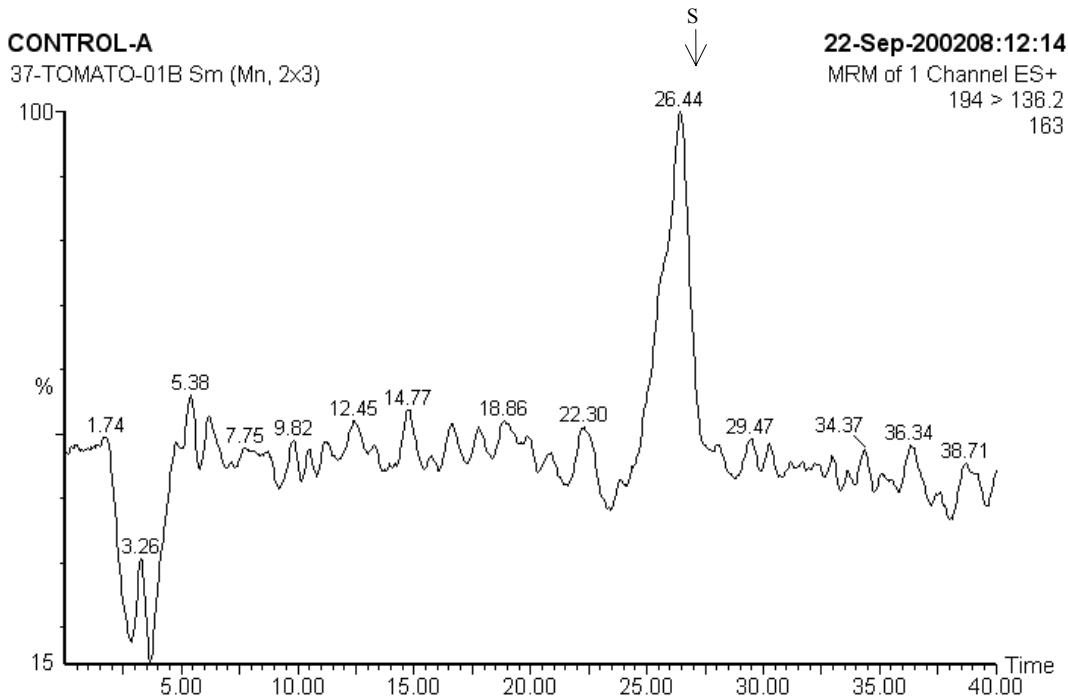


1. 5.0 ng standard, CGA-37913 (Racemic Standard)

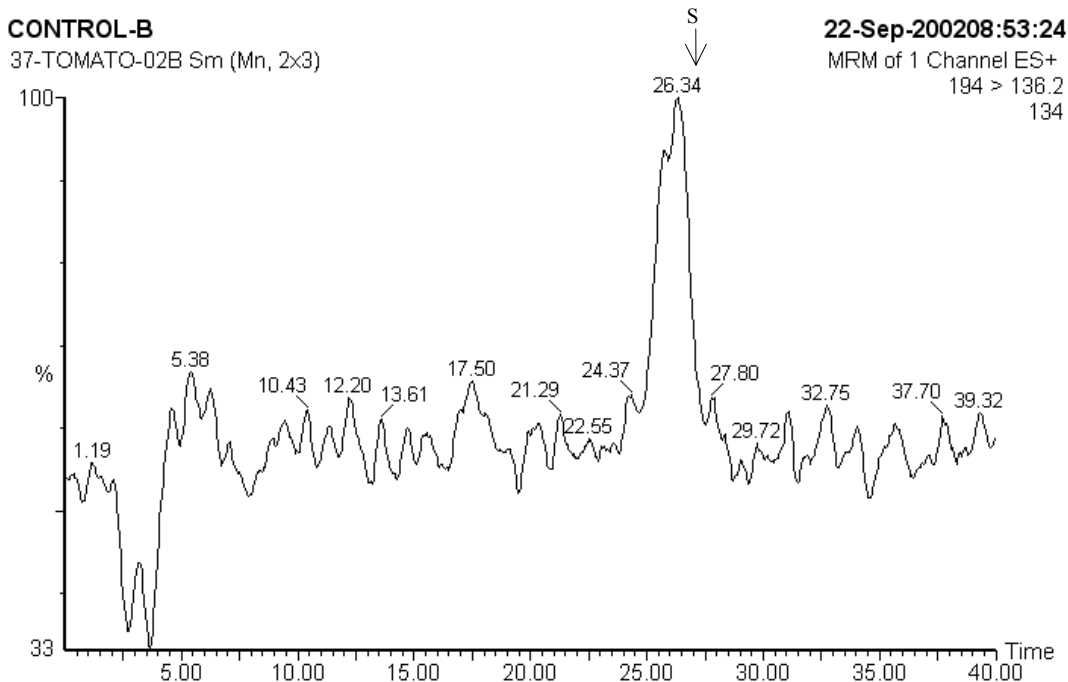


2. 5.0 ng standard, SYN506357

FIGURE 6. CHROMATOGRAMS SYN506357 IN TOMATO (CONT.)

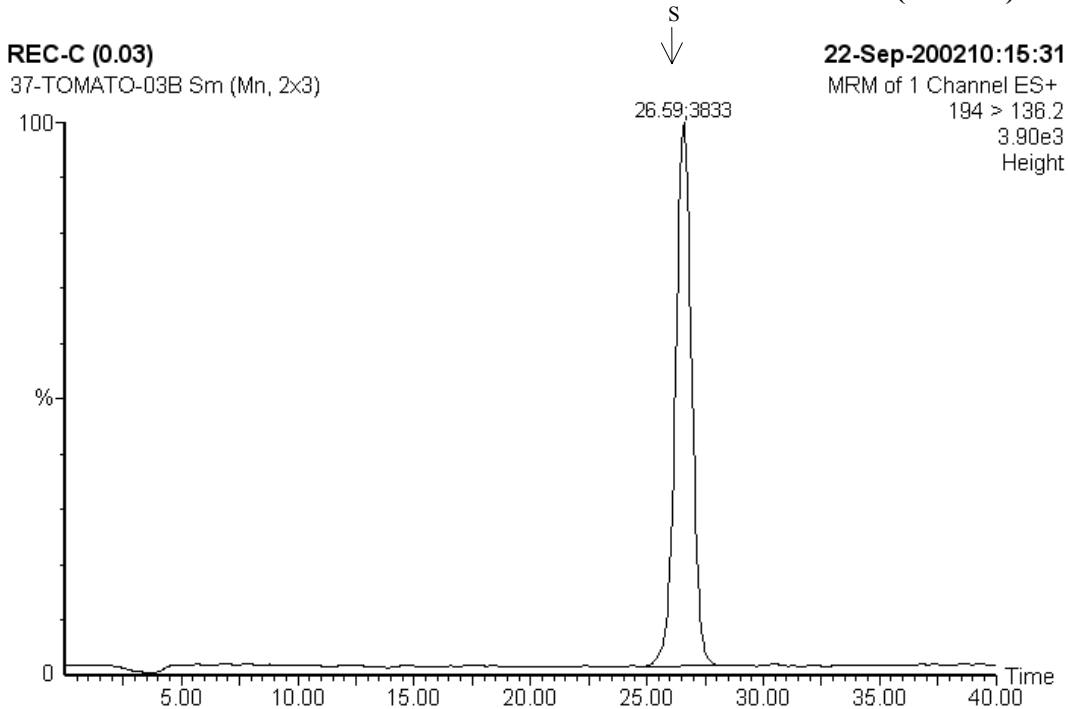


3. control, 3.53 mg injected, 0.00 ng found, <0.03 ppm

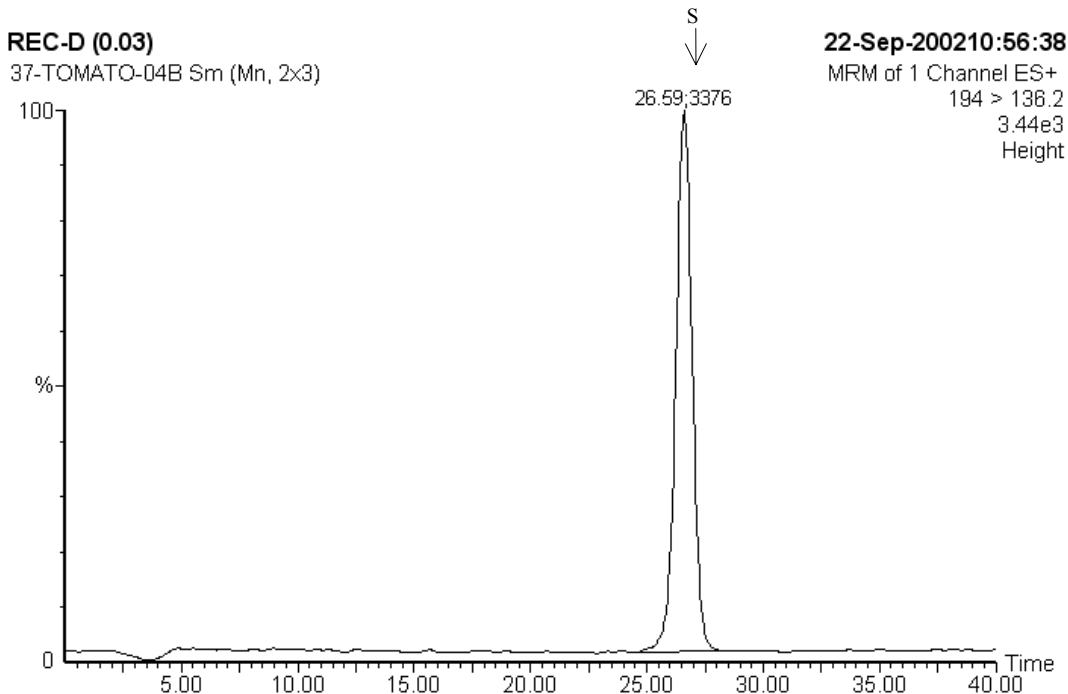


4. control, 3.53 mg injected, 0.00 ng found, <0.03 ppm

FIGURE 6. CHROMATOGRAMS SYN506357 IN TOMATO (CONT.)



5. control + 0.03 ppm SYN506357, 3.53 mg injected, 0.130 ng found, 0.037 ppm, 123% recovery

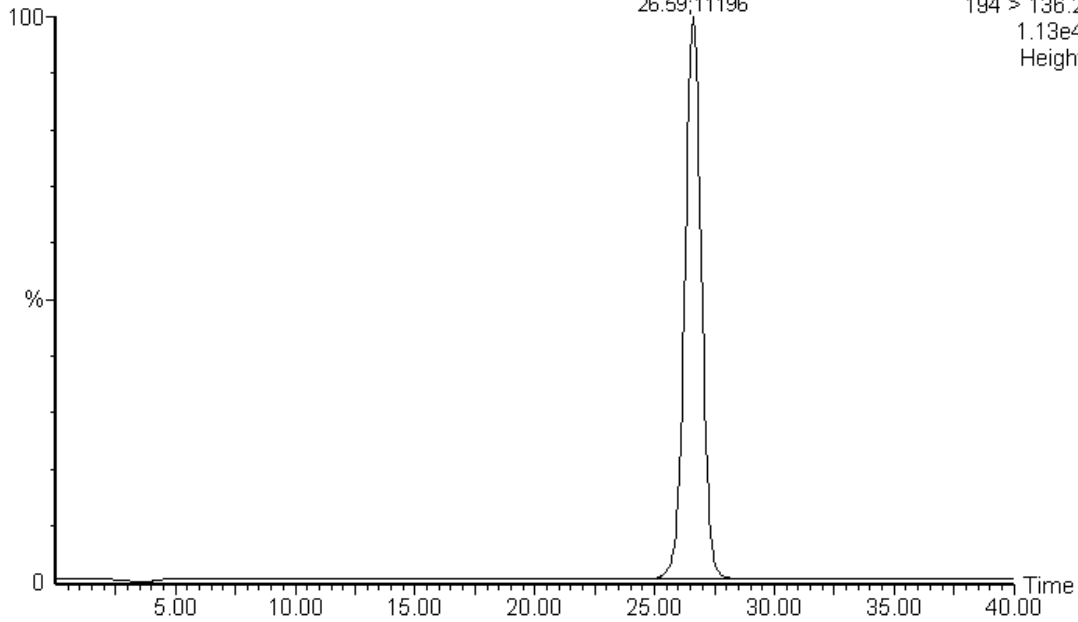


6. control + 0.03 ppm SYN506357, 3.53 mg injected, 0.116 ng found, 0.033 ppm, 110% recovery

FIGURE 6. CHROMATOGRAMS SYN506357 IN TOMATO (CONT.)

REC-E (0.1)

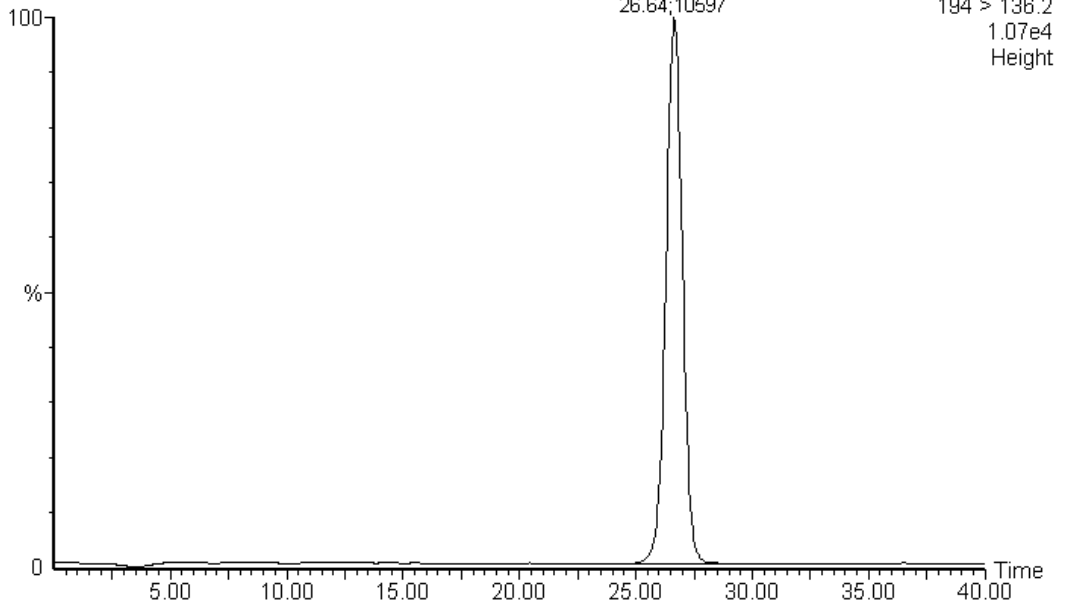
37-TOMATO-05B Sm (Mn, 2x3)



7. control + 0.10 ppm SYN506357, 3.53 mg injected, 0.359 ng found, 0.102 ppm, 102% recovery

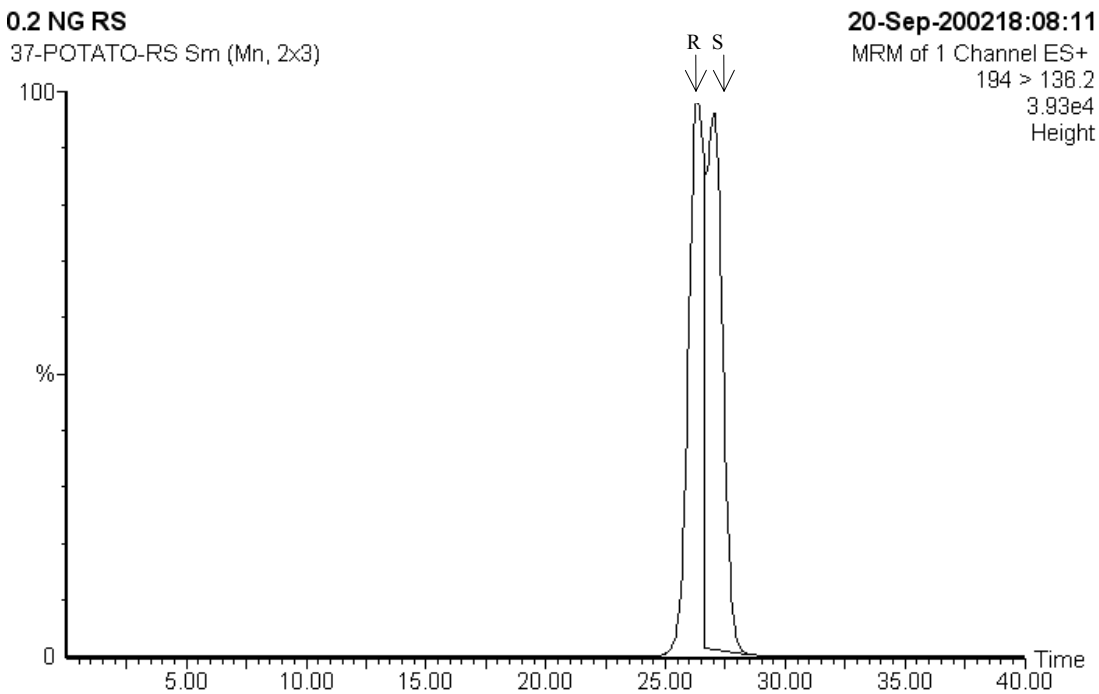
REC-F (0.1)

37-TOMATO-06B Sm (Mn, 2x3)

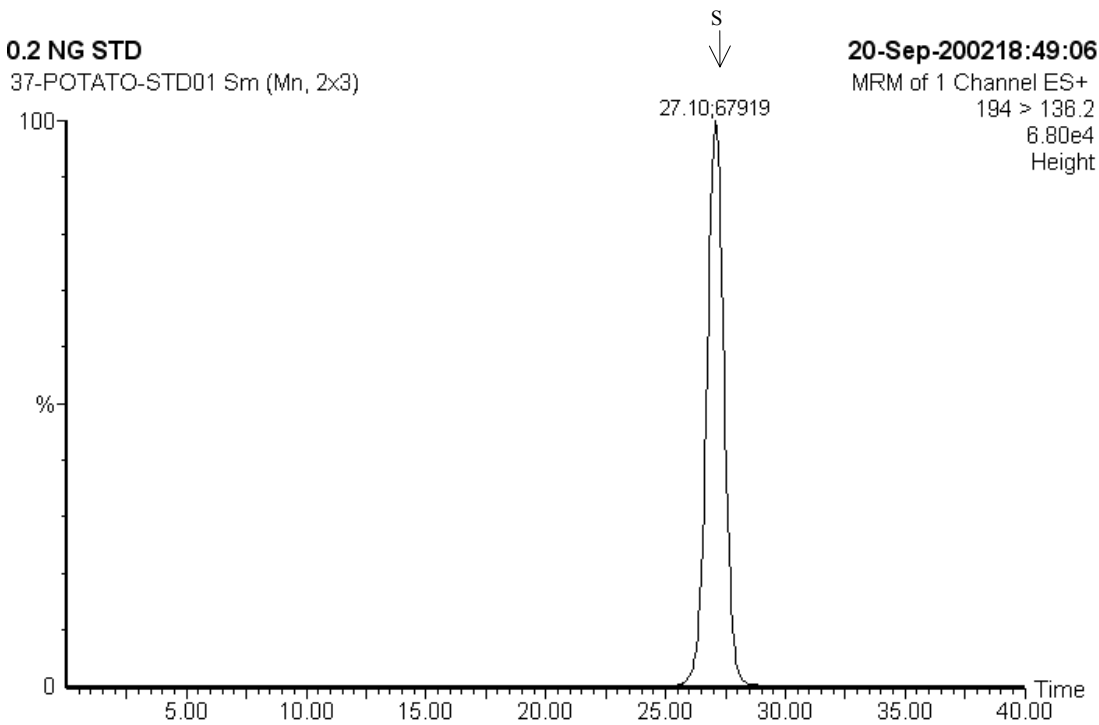


8. control + 0.10 ppm SYN506357, 3.53 mg injected, 0.340 ng found, 0.096 ppm, 96.3% recovery

FIGURE 7. CHROMATOGRAMS SYN506357 IN POTATO

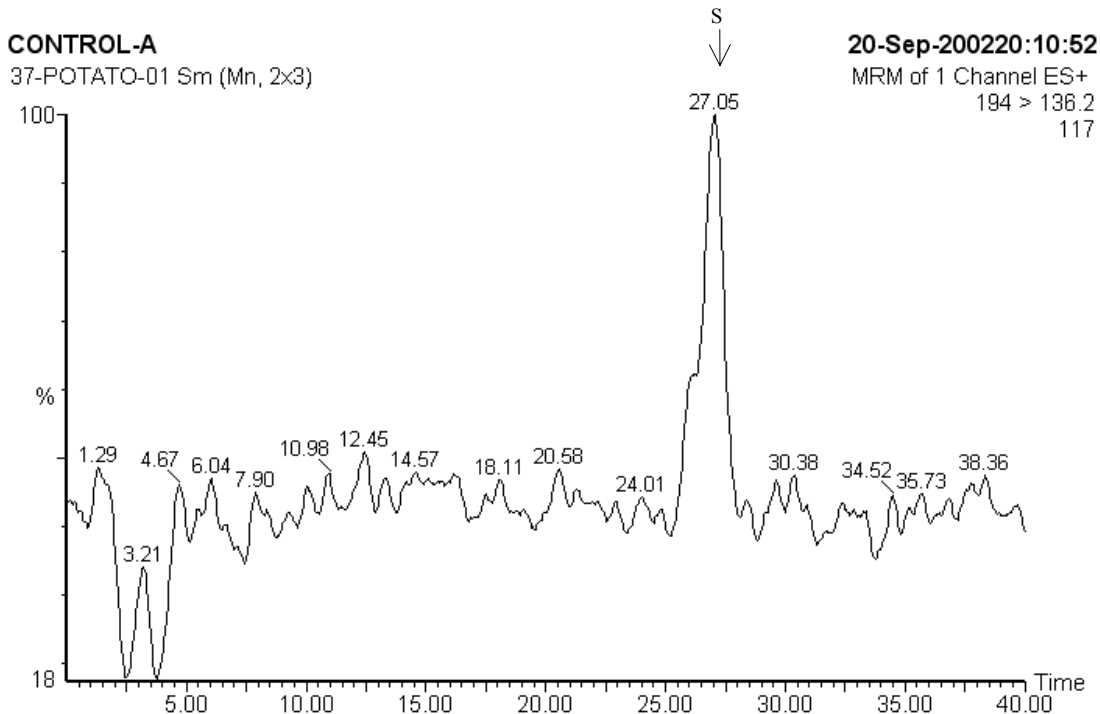


1. 5.0 ng standard, SYN506357 (Racemic Standard)

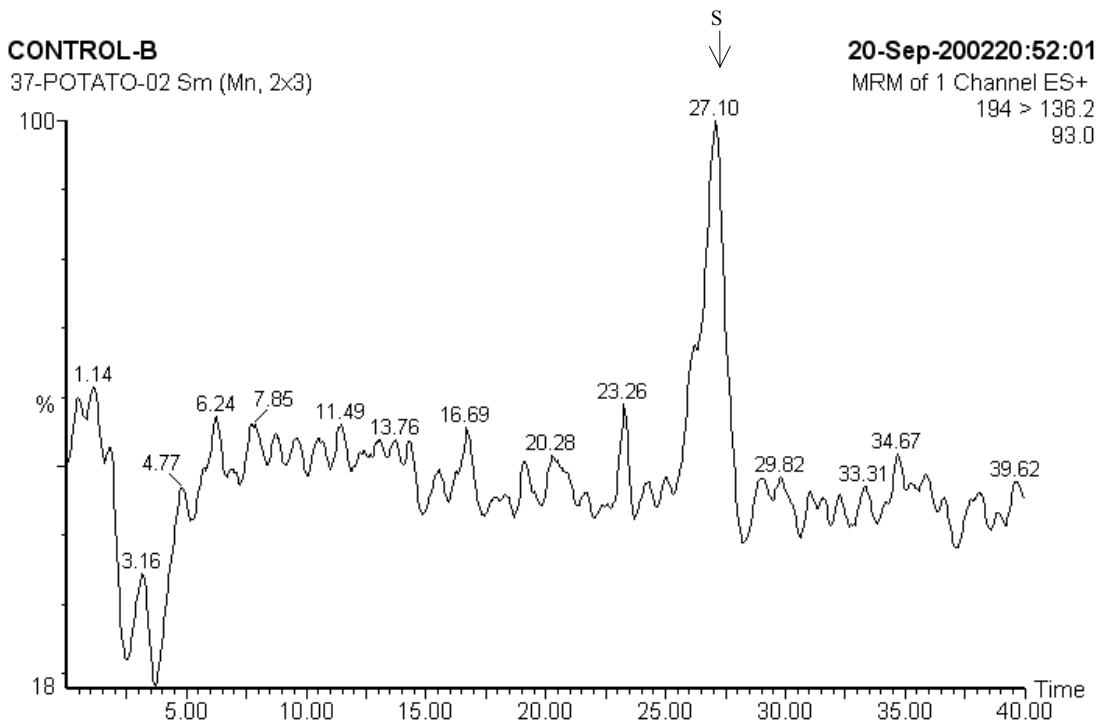


2. 5.0 ng standard, SYN506357

FIGURE 7. CHROMATOGRAMS SYN506357 IN POTATO (CONT.)

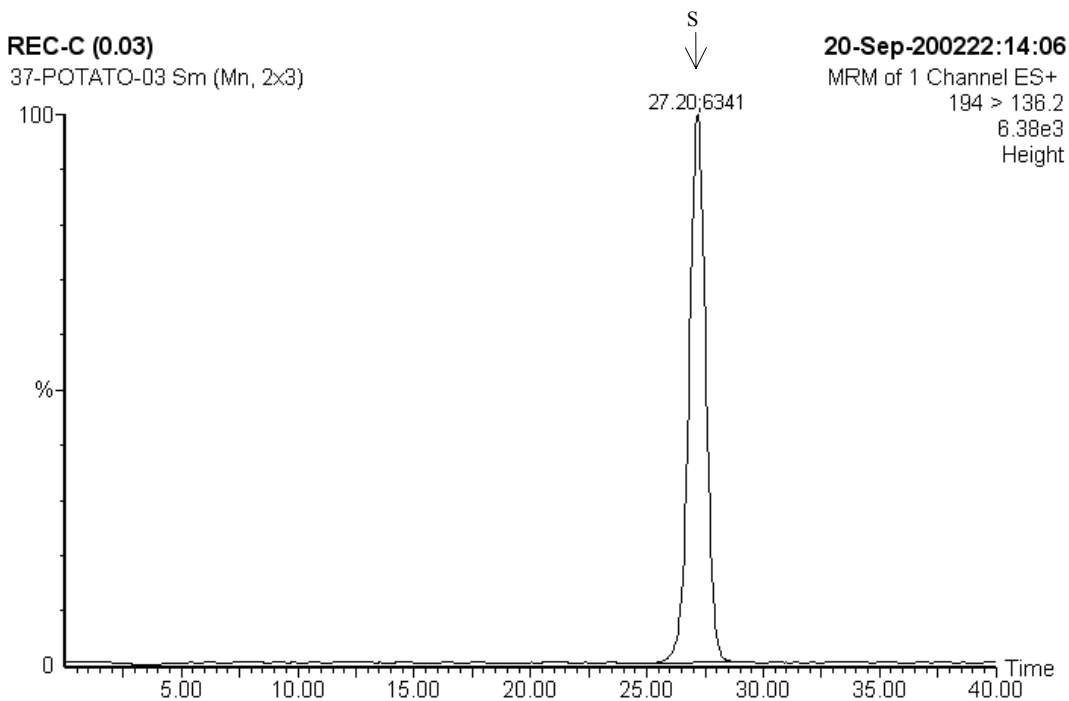


3. control, 14.1 mg injected, 0.00 ng found, <0.03 ppm

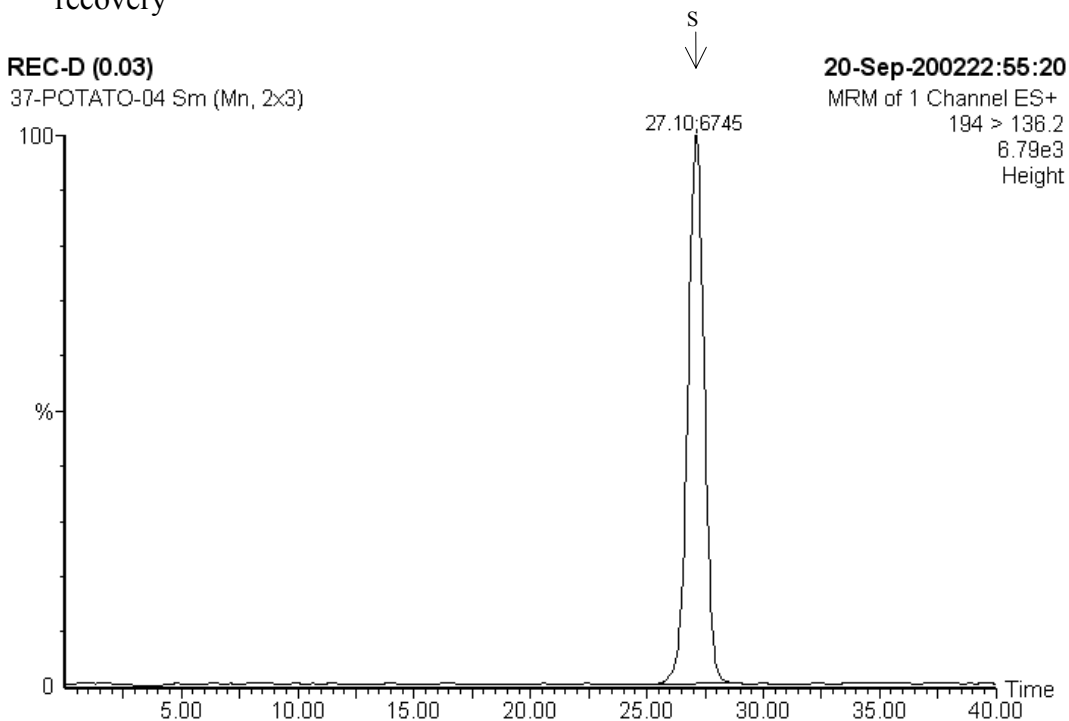


4. control, 14.1 mg injected, 0.00 ng found, <0.03 ppm

FIGURE 7. CHROMATOGRAMS SYN506357 IN POTATO (CONT.)

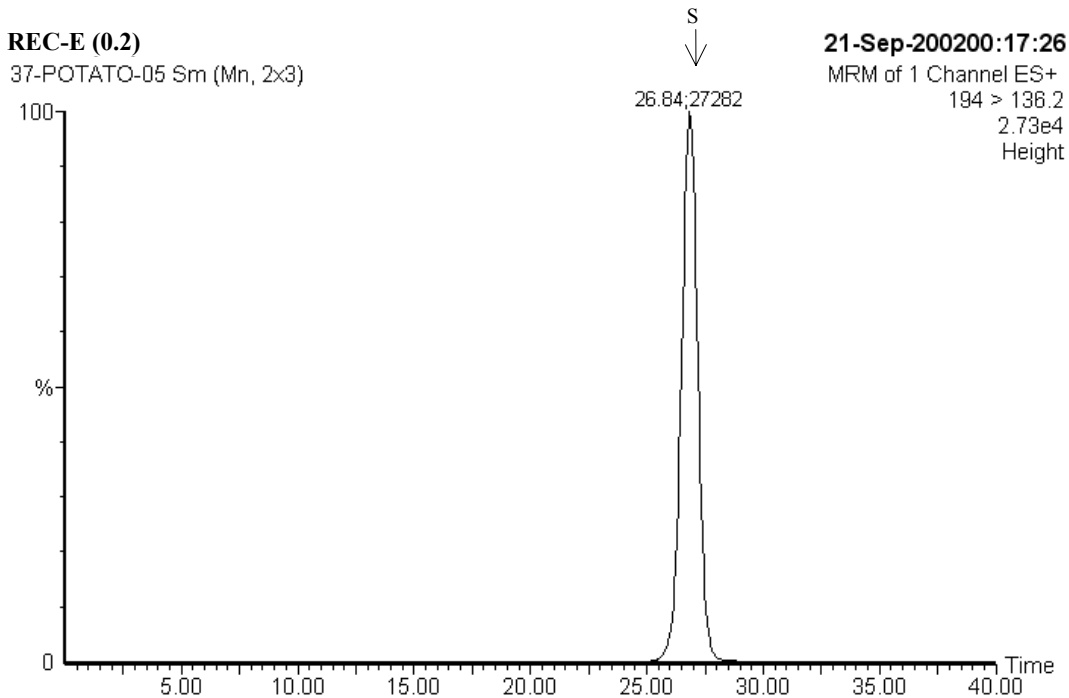


5. control + 0.03 ppm SYN506357, 14.1 mg injected, 0.345 ng found, 0.024 ppm, 81.4% recovery

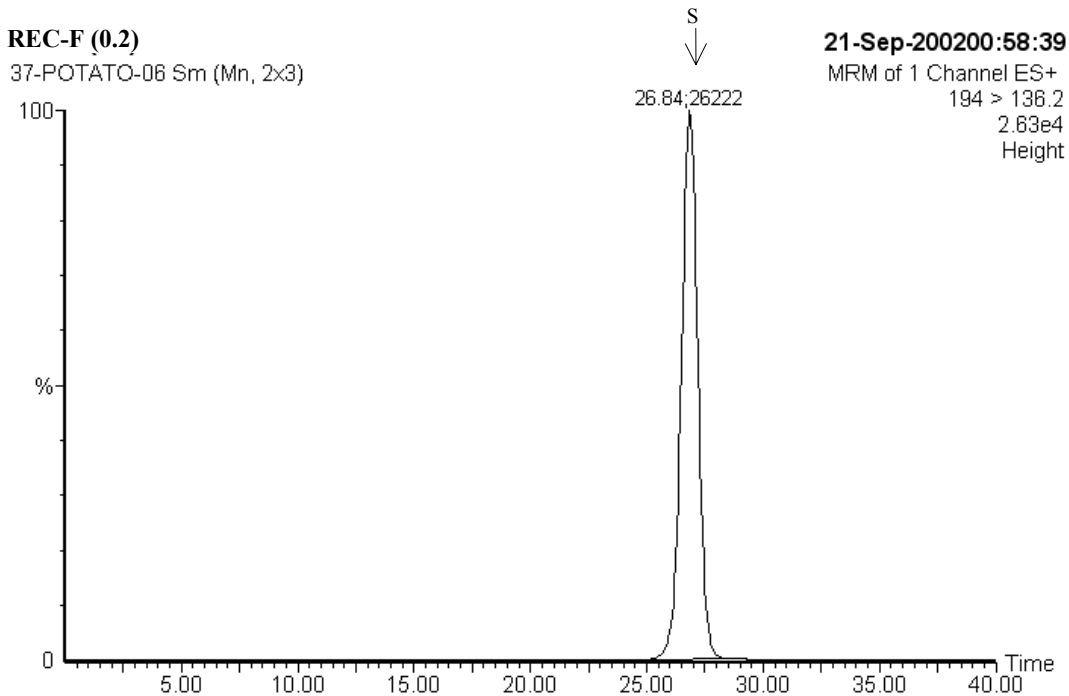


6. control + 0.03 ppm SYN506357, 14.1 mg injected, 0.369 ng found, 0.026 ppm, 87.1% recovery

FIGURE 7. CHROMATOGRAMS SYN506357 IN POTATO (CONT.)

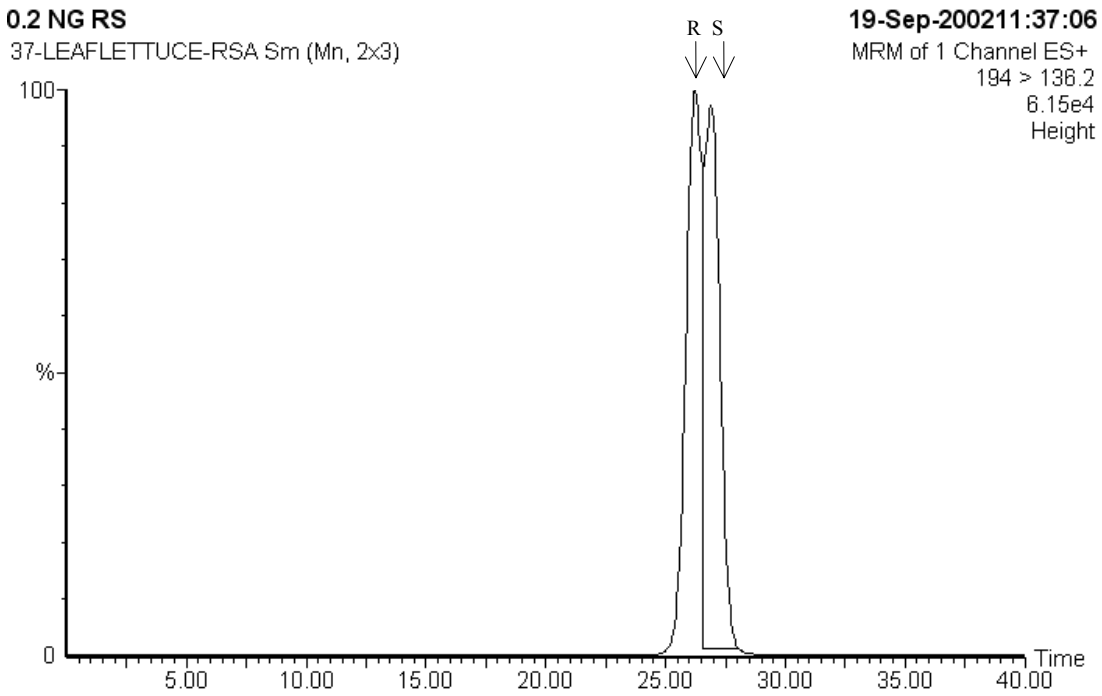


7. control + 0.20 ppm SYN506357, 7.07 mg injected, 1.60 ng found, 0.226 ppm, 113% recovery

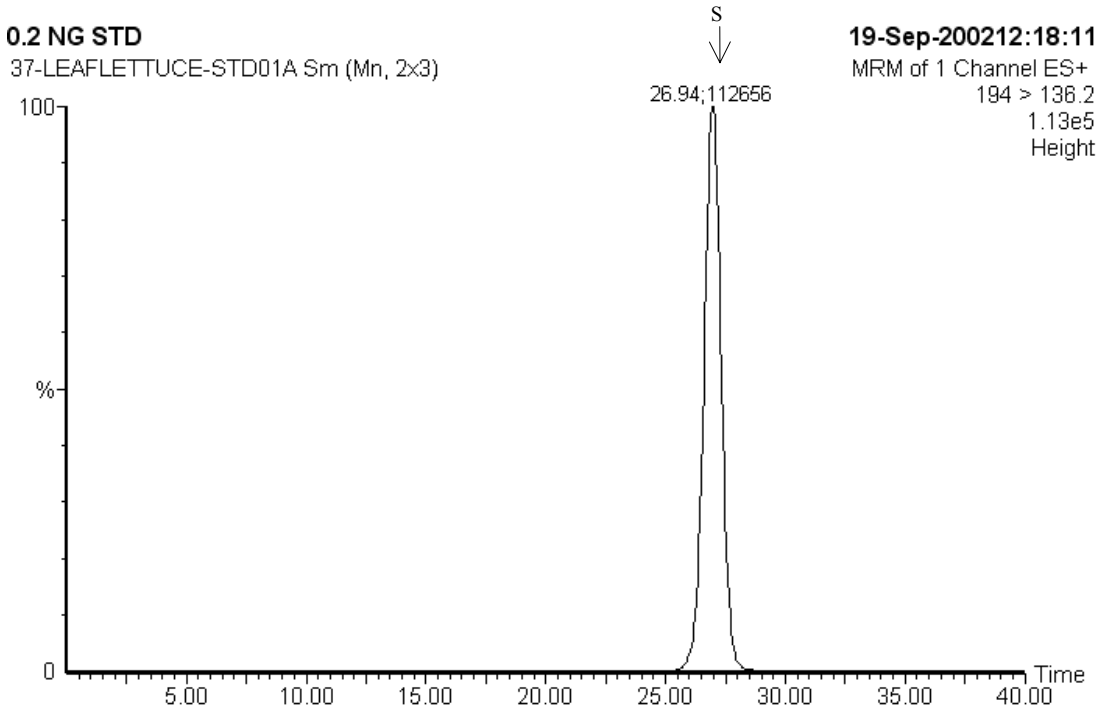


8. control + 0.20 ppm SYN506357, 7.07 mg injected, 1.53 ng found, 0.217 ppm, 108% recovery

FIGURE 8. CHROMATOGRAMS SYN506357 IN LEAF LETTUCE

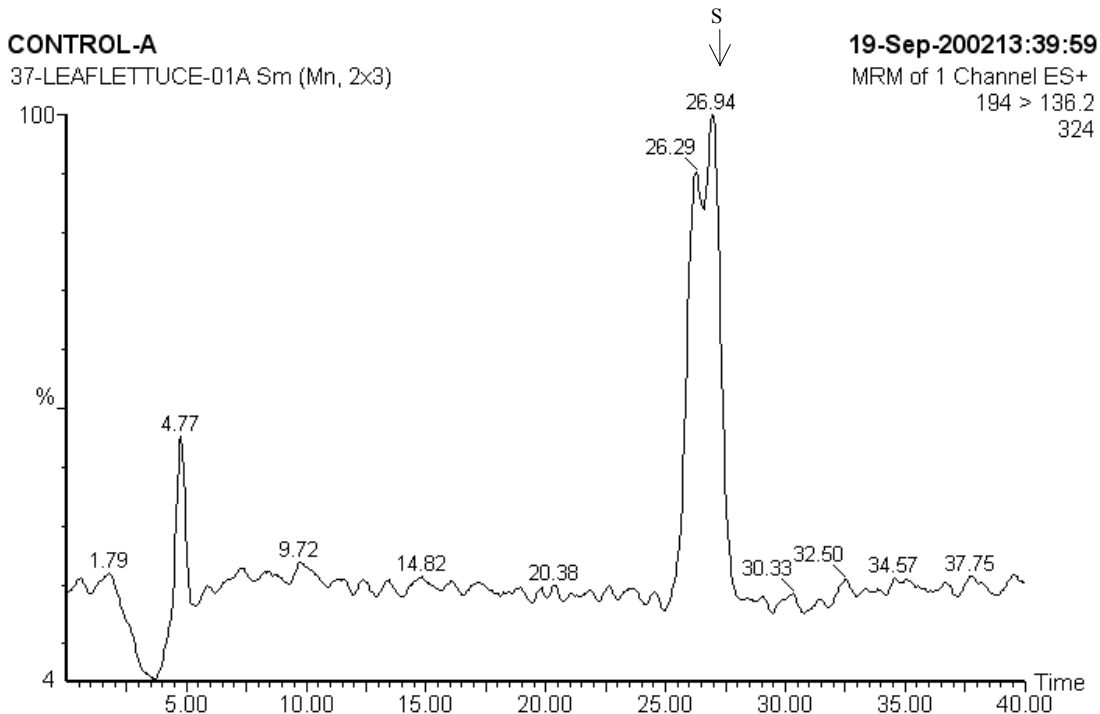


1. 5.0 ng standard, CGA-37913 (Racemic Standard)

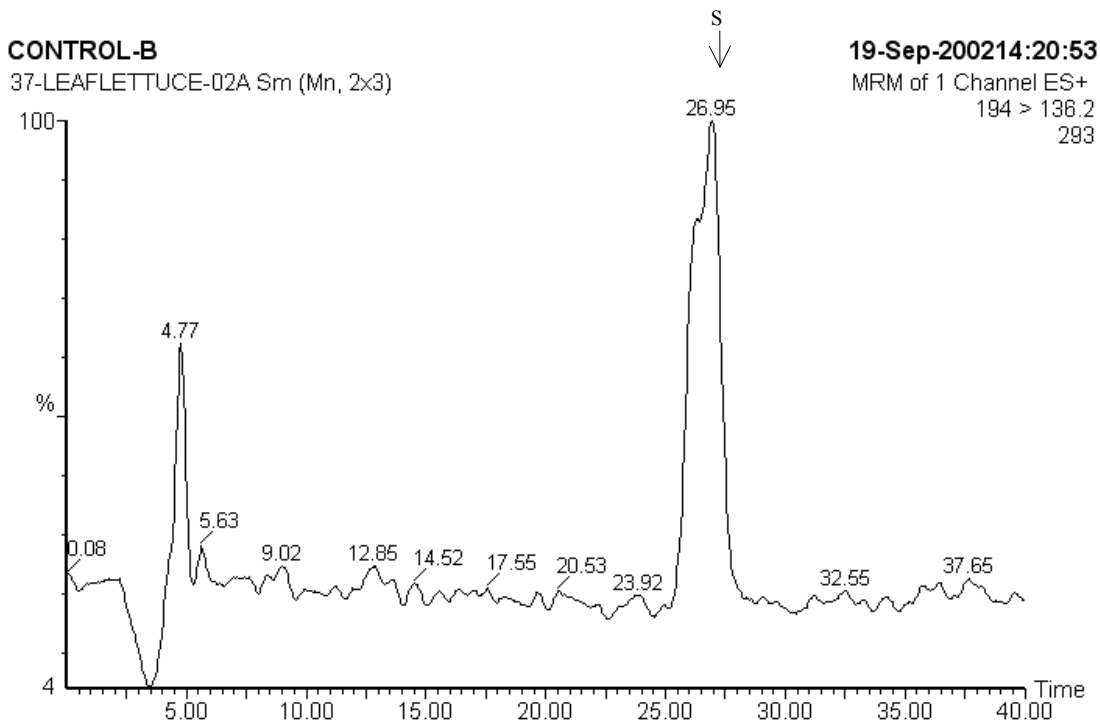


2. 5.0 ng standard, SYN506357

FIGURE 8. CHROMATOGRAMS SYN506357 IN LEAF LETTUCE (CONT.)

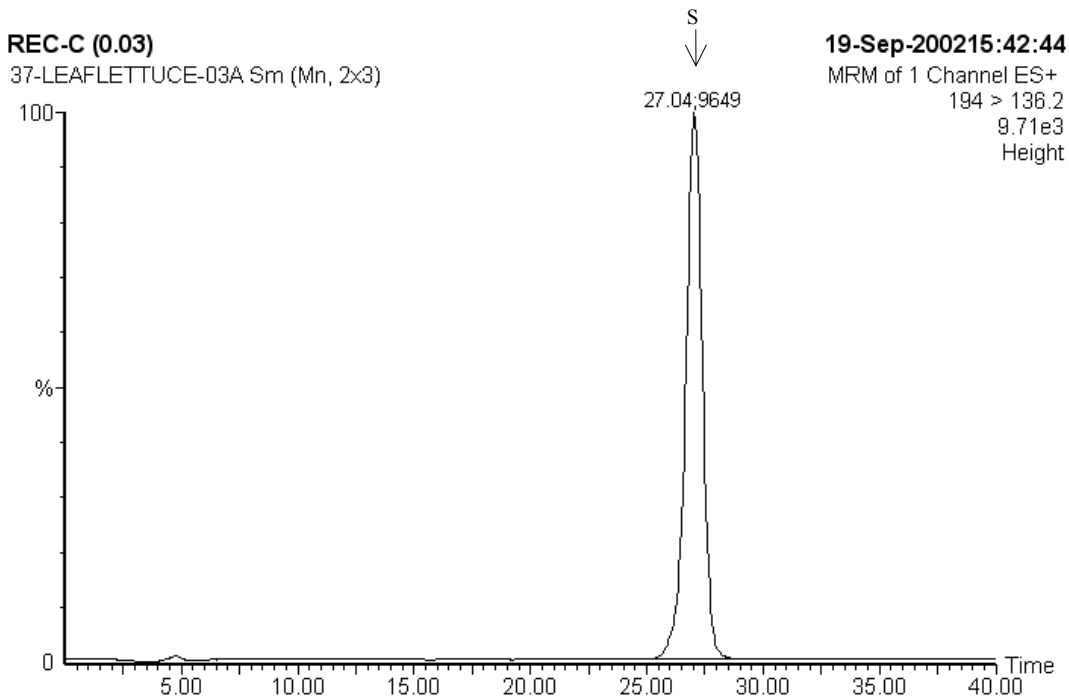


3. control, 14.0 mg injected, 0.00 ng found, <0.03 ppm

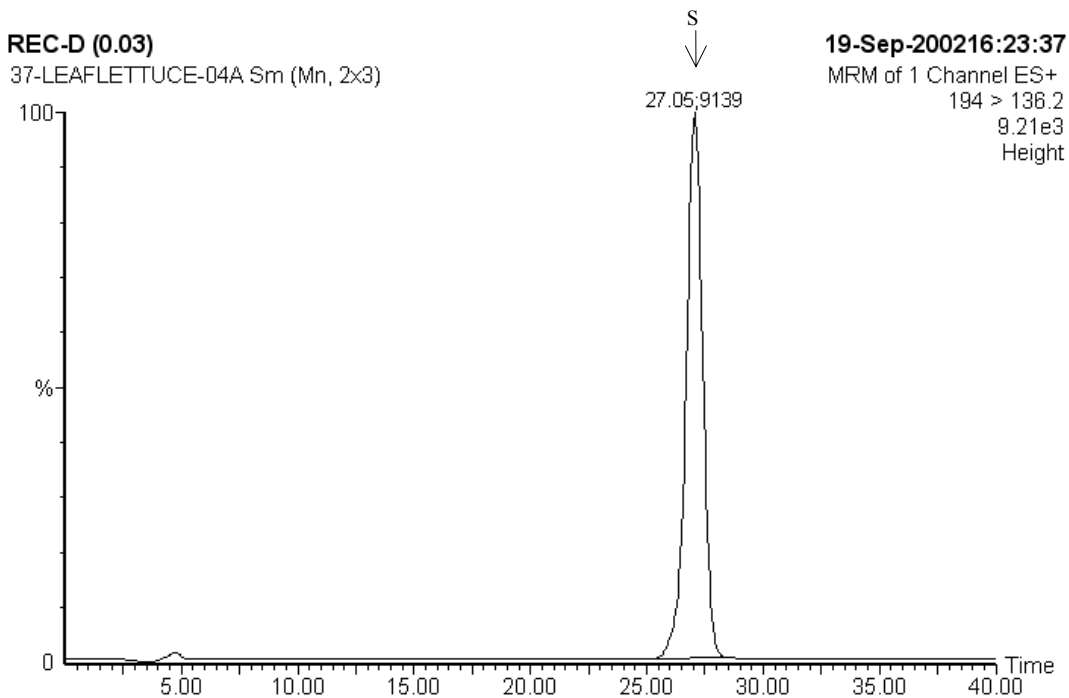


4. control, 14.0 mg injected, 0.00 ng found, <0.03 ppm

FIGURE 8. CHROMATOGRAMS SYN506357 IN LEAF LETTUCE (CONT.)

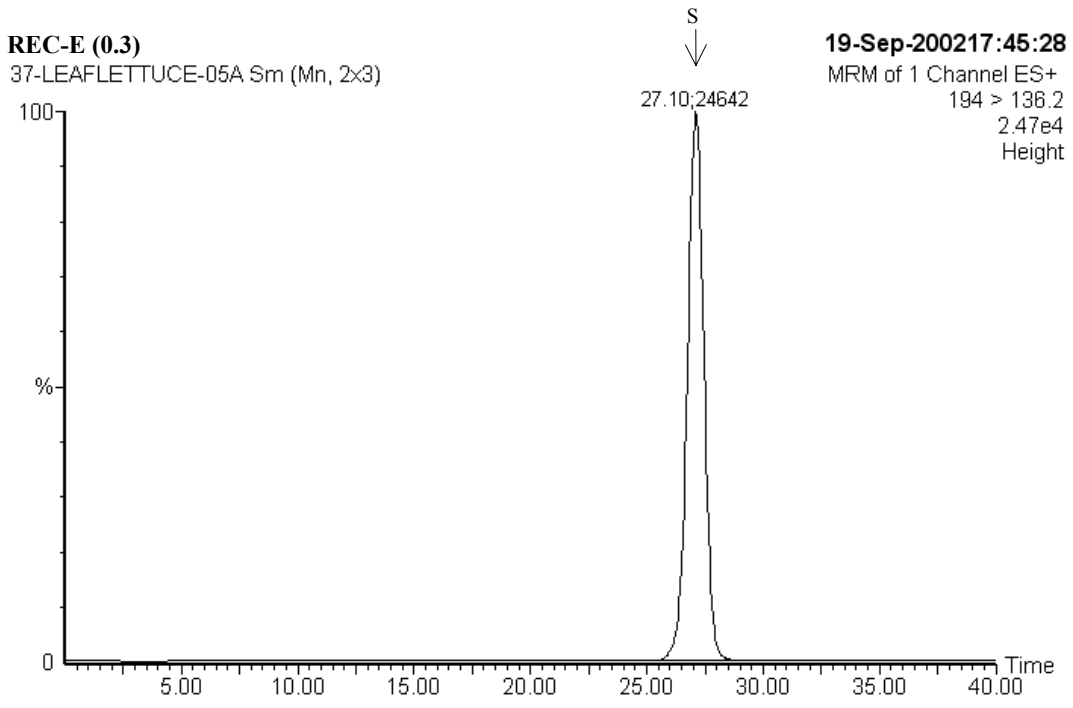


5. control + 0.03 ppm SYN506357, 14.0 mg injected, 0.419 ng found, 0.030 ppm, 99.8% recovery

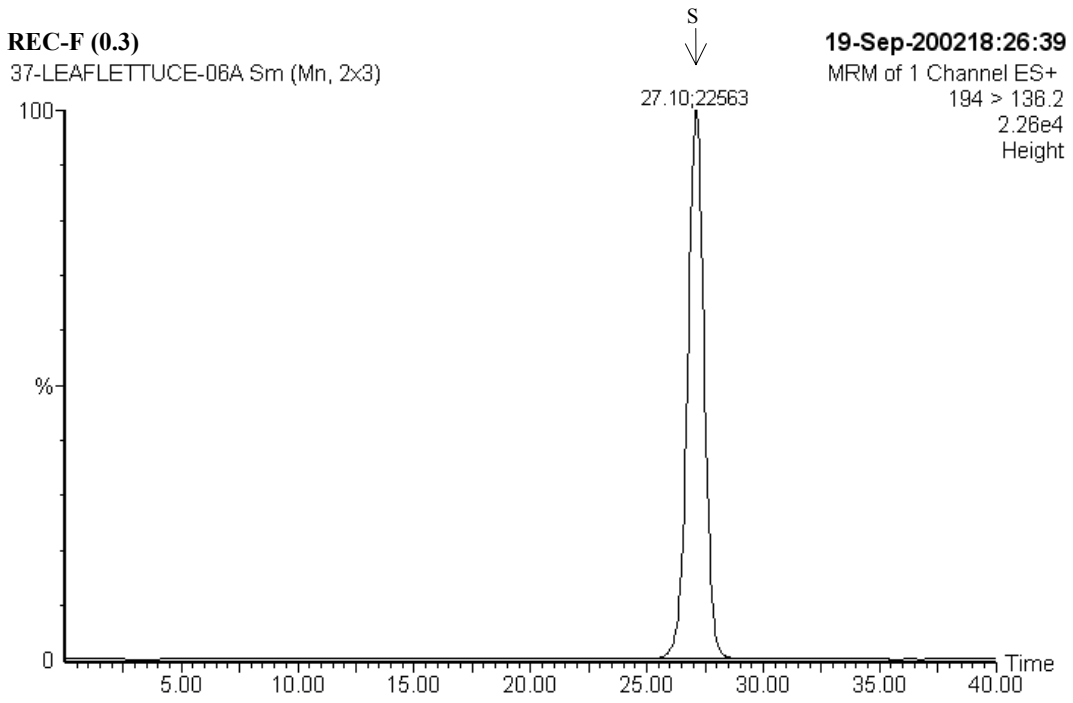


6. control + 0.03 ppm SYN506357, 14.0 mg injected, 0.396 ng found, 0.028 ppm, 94.4% recovery

FIGURE 8. CHROMATOGRAMS SYN506357 IN LEAF LETTUCE (CONT.)

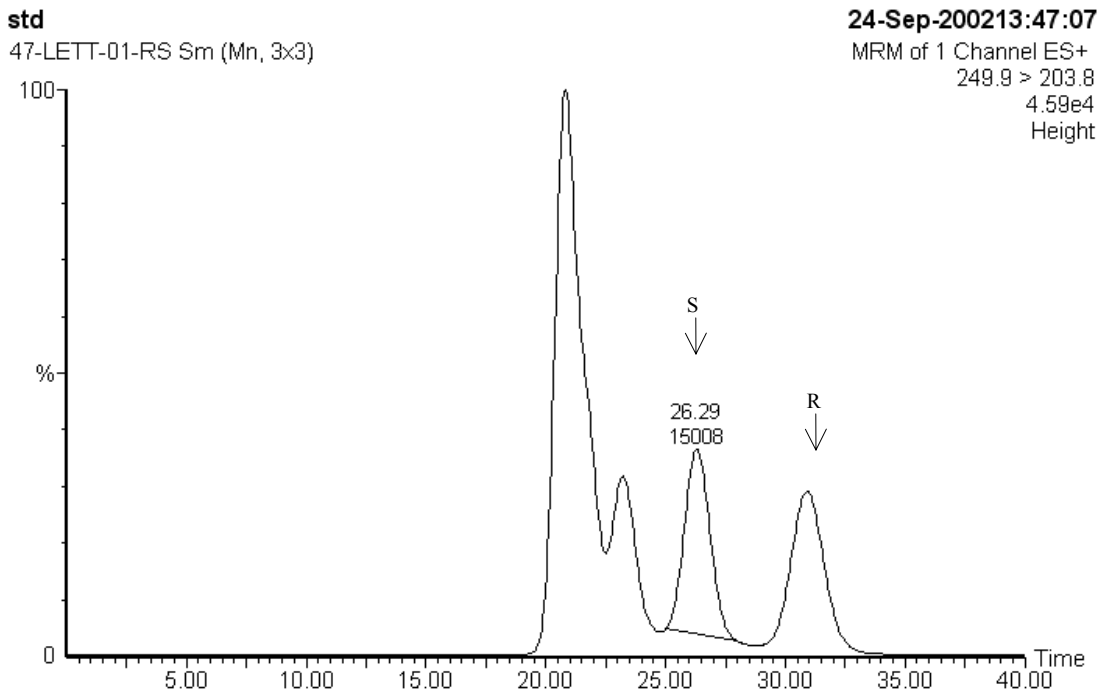


7. control + 0.30 ppm SYN506357, 3.50 mg injected, 1.08 ng found, 0.310 ppm, 103% recovery

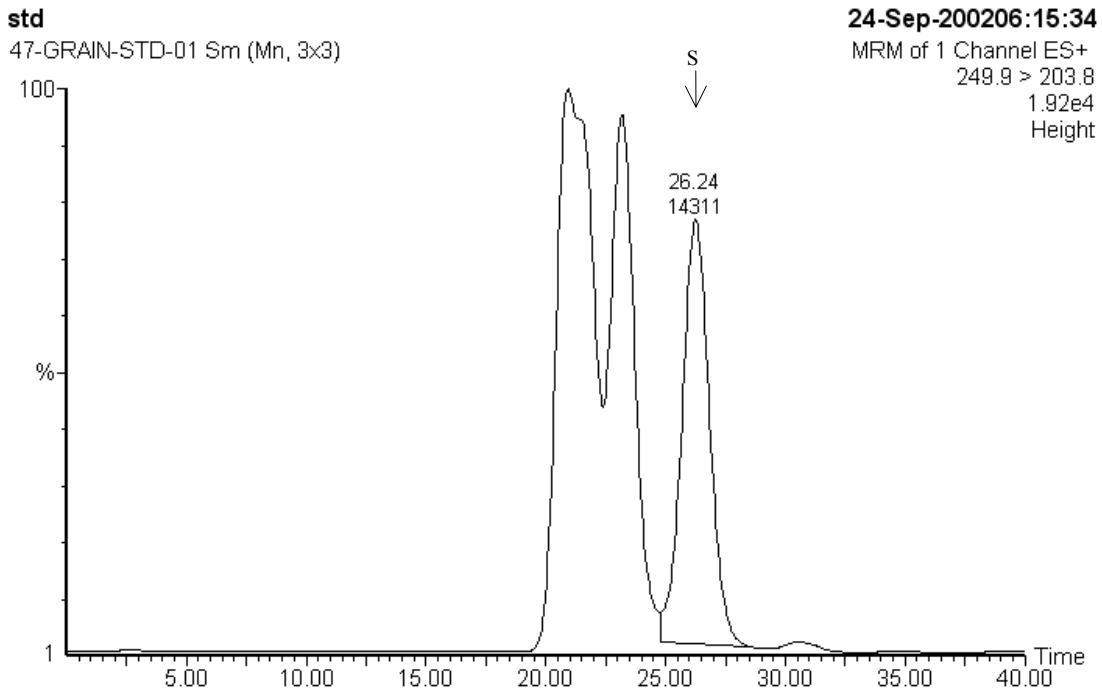


8. control + 0.30 ppm SYN506357, 3.50 mg injected, 0.991 ng found, 0.284 ppm, 94.5% recovery

FIGURE 9. CHROMATOGRAMS SYN508500 IN CORN GRAIN



1. 1.25 ng standard, CGA-49751 (Racemic Standard)



2. 1.25 ng standard, SYN508500

FIGURE 9. CHROMATOGRAMS SYN508500 IN CORN GRAIN (CONT.)

CONTROL

47-GRAIN-01 Sm (Mn, 3x3)

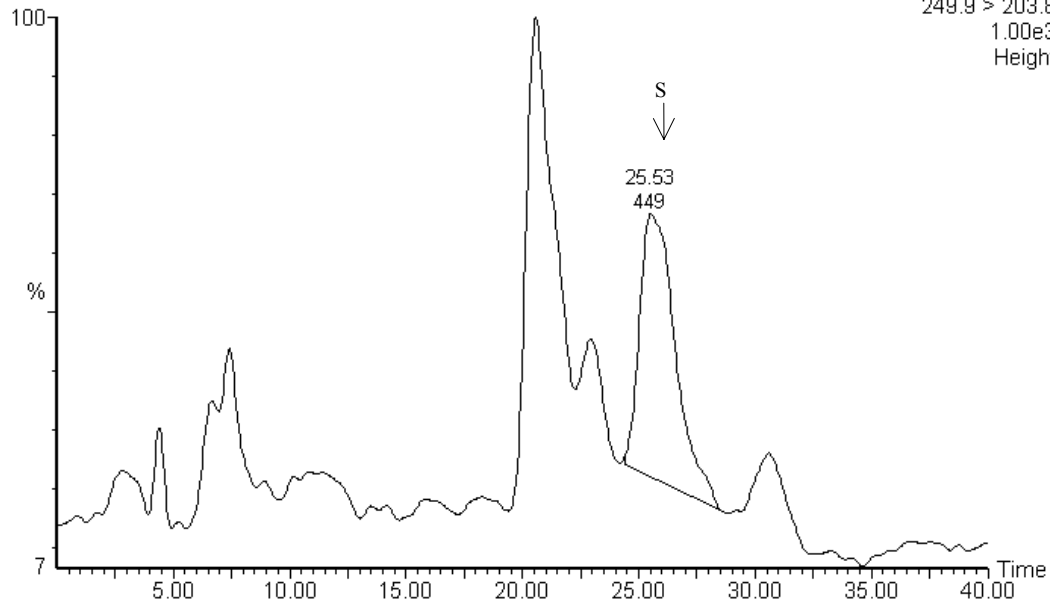
24-Sep-200207:37:27

MRM of 1 Channel ES+

249.9 > 203.8

1.00e3

Height



3. control, 9.62 mg injected, 0.02 ng found, <0.05 ppm

CONTROL

47-GRAIN-02 Sm (Mn, 3x3)

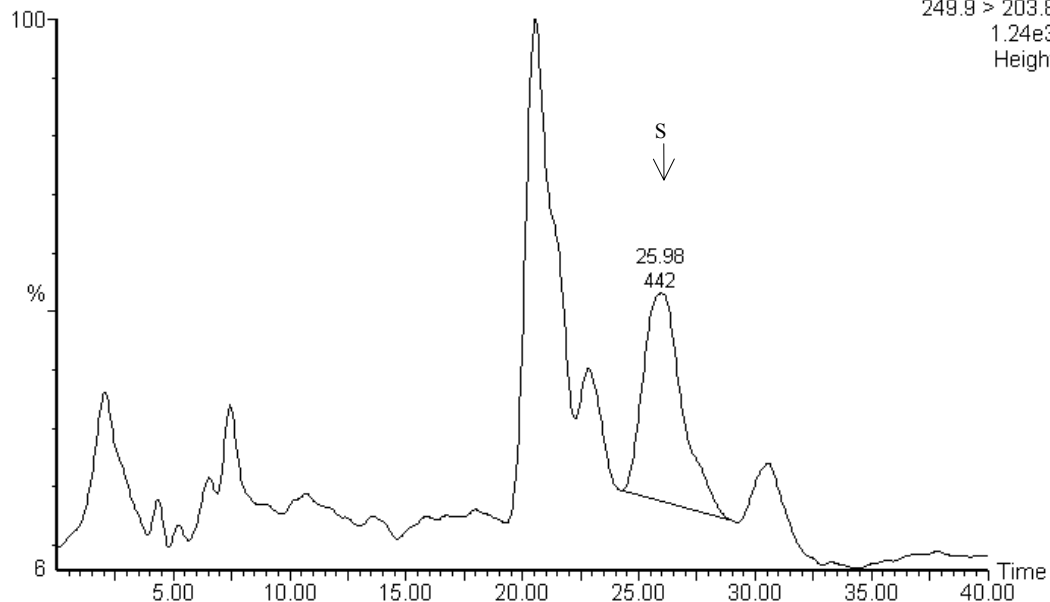
24-Sep-200208:18:37

MRM of 1 Channel ES+

249.9 > 203.8

1.24e3

Height



4. control, 9.62 mg injected, 0.01 ng found, <0.05 ppm

FIGURE 9. CHROMATOGRAMS SYN508500 IN CORN GRAIN (CONT.)

0.05 PPM REC

47-GRAIN-03 Sm (Mn, 3x3)

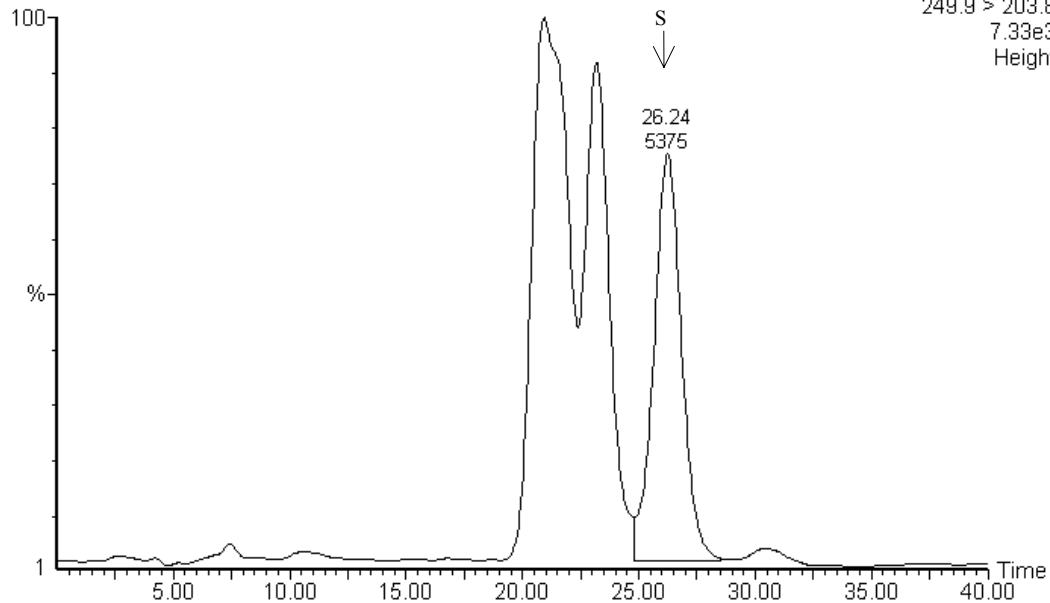
24-Sep-200209:40:43

MRM of 1 Channel ES+

249.9 > 203.8

7.33e3

Height



5. control + 0.05 ppm SYN508500, 9.62 mg injected, 0.449 ng found, 0.047 ppm, 90.3% recovery

0.05 PPM REC

47-GRAIN-04 Sm (Mn, 3x3)

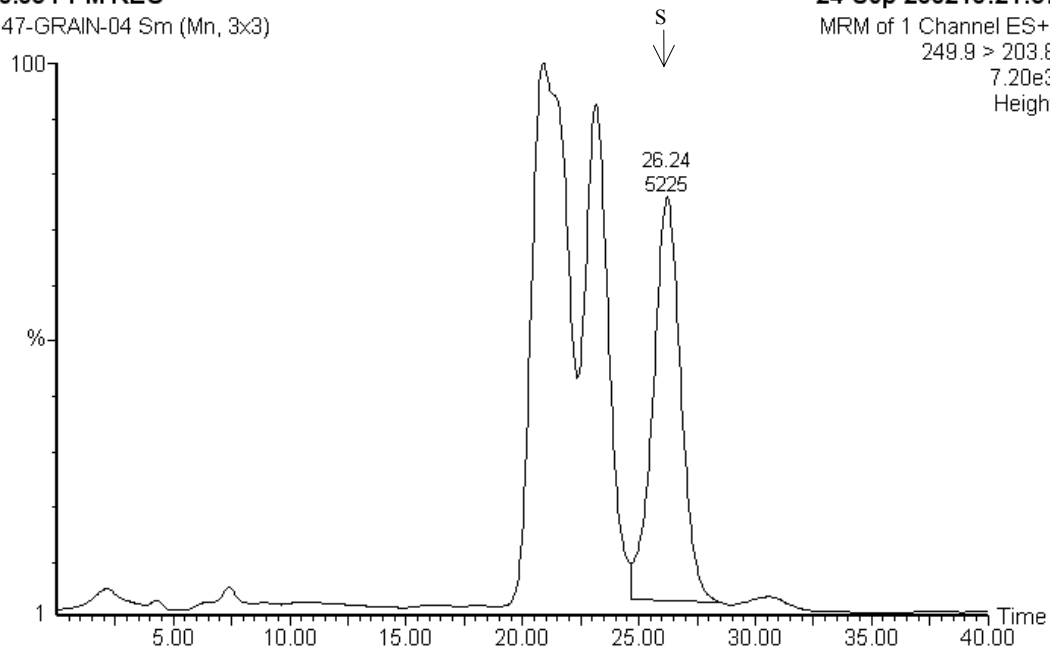
24-Sep-200210:21:57

MRM of 1 Channel ES+

249.9 > 203.8

7.20e3

Height



6. control + 0.05 ppm SYN508500, 9.62 mg injected, 0.436 ng found, 0.045 ppm, 87.5% recovery

FIGURE 9. CHROMATOGRAMS SYN508500 IN CORN GRAIN (CONT.)

0.1 PPM REC

47-GRAIN-05 Sm (Mn, 3x3)

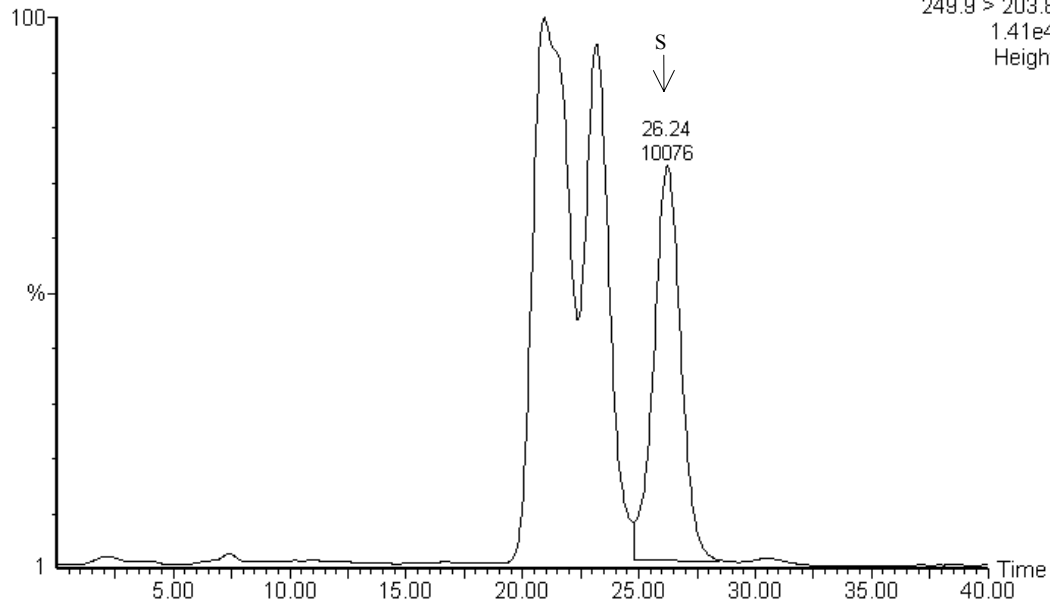
24-Sep-200211:02:53

MRM of 1 Channel ES+

249.9 > 203.8

1.41e4

Height



7. control + 0.10 ppm SYN508500, 9.62 mg injected, 0.864 ng found, 0.090 ppm, 88.2% recovery

0.1 PPM REC

47-GRAIN-06 Sm (Mn, 3x3)

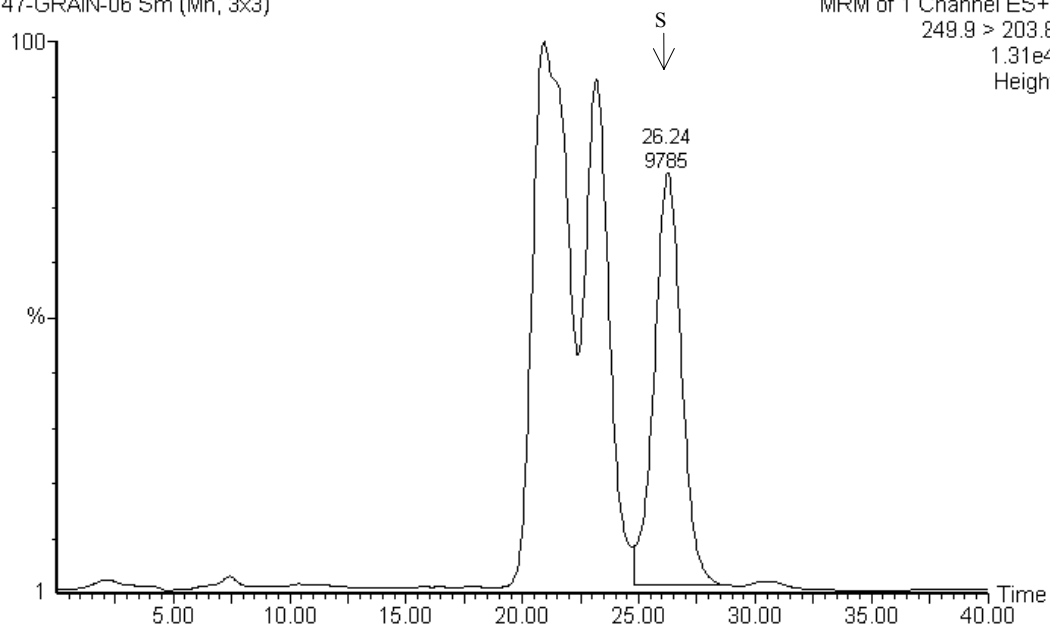
24-Sep-200211:43:49

MRM of 1 Channel ES+

249.9 > 203.8

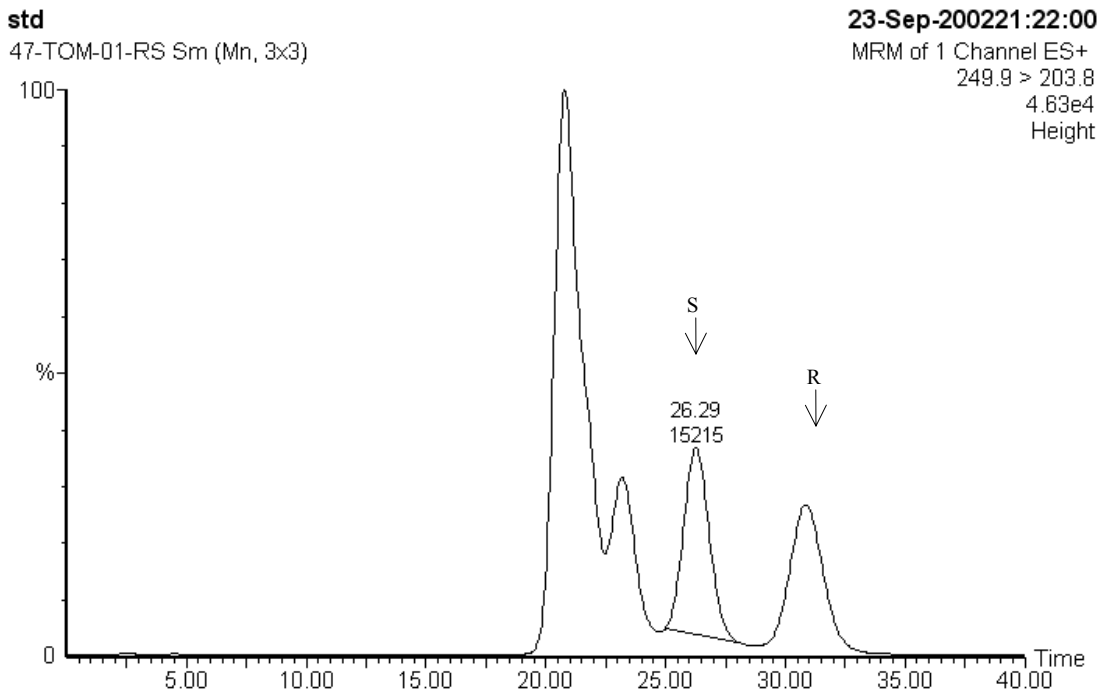
1.31e4

Height

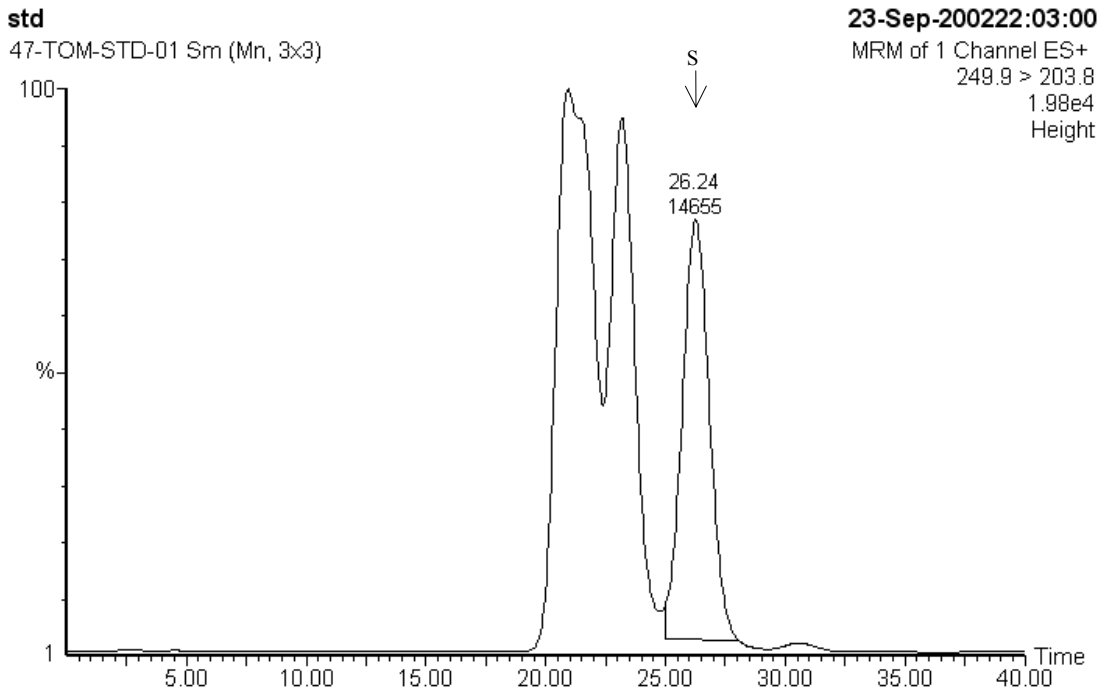


8. control + 0.10 ppm SYN508500, 9.62 mg injected, 0.838 ng found, 0.087 ppm, 85.6% recovery

FIGURE 10. CHROMATOGRAMS SYN508500 IN TOMATO



1. 1.25 ng standard, CGA-49751 (Racemic Standard)



2 1.25 ng standard, SYN508500

FIGURE 10. CHROMATOGRAMS SYN508500 IN TOMATO (CONT.)

CONTROL

47-TOM-01 Sm (Mn, 3x3)

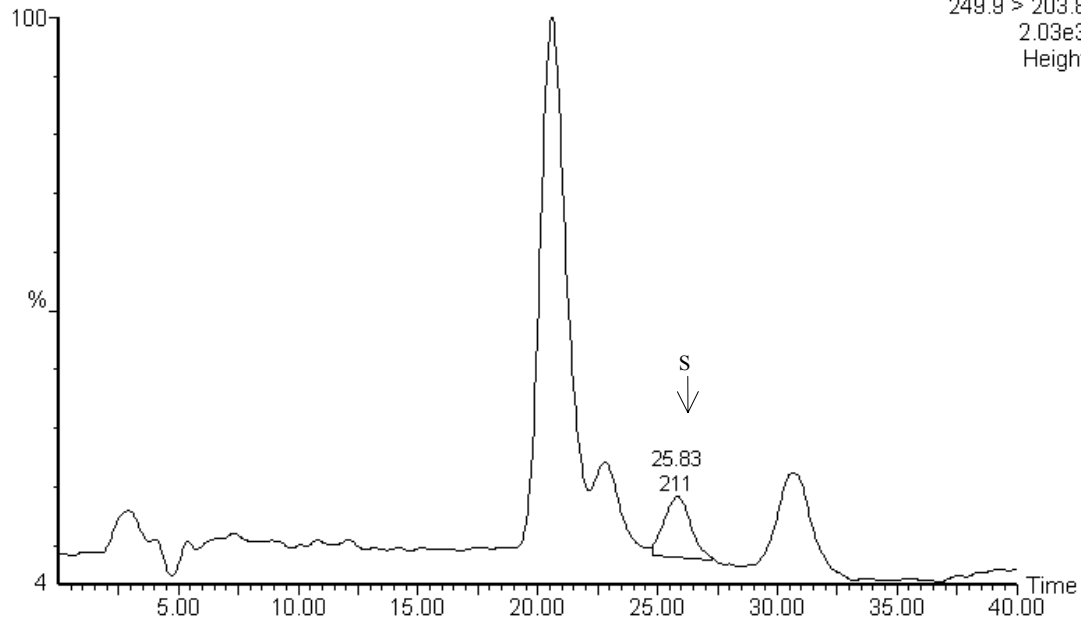
23-Sep-200223:24:54

MRM of 1 Channel ES+

249.9 > 203.8

2.03e3

Height



3. control, 8.83 mg injected, 0.00 ng found, <0.05 ppm

CONTROL

47-TOM-02 Sm (Mn, 3x3)

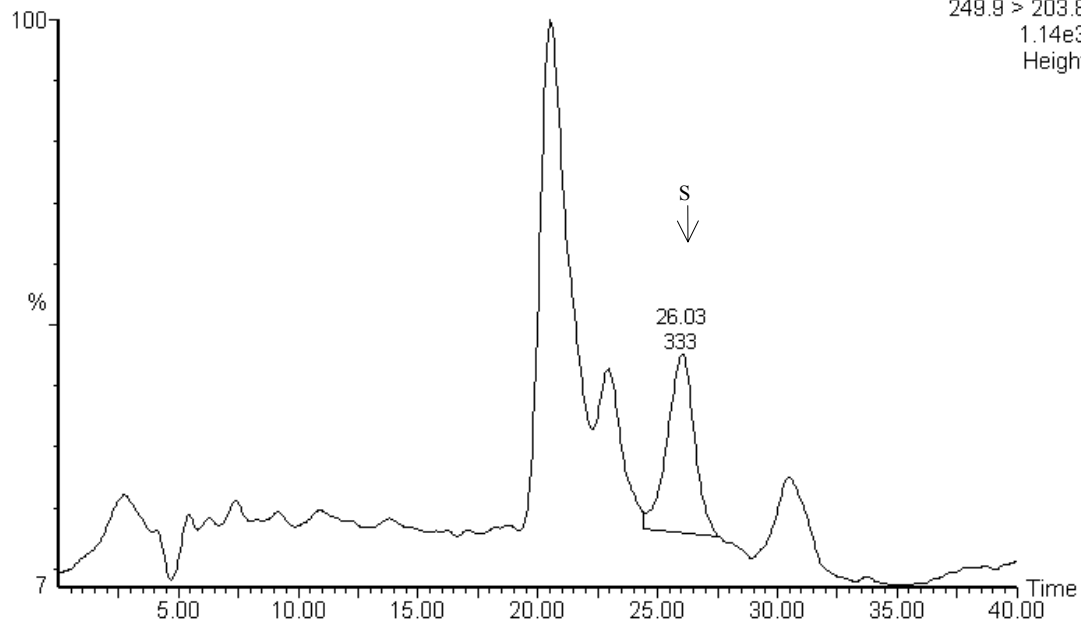
24-Sep-200200:06:05

MRM of 1 Channel ES+

249.9 > 203.8

1.14e3

Height



4. control, 8.83 mg injected, 0.00 ng found, <0.05 ppm

FIGURE 10. CHROMATOGRAMS SYN508500 IN TOMATO (CONT.)

0.05 PPM REC

47-TOM-03 Sm (Mn, 3x3)

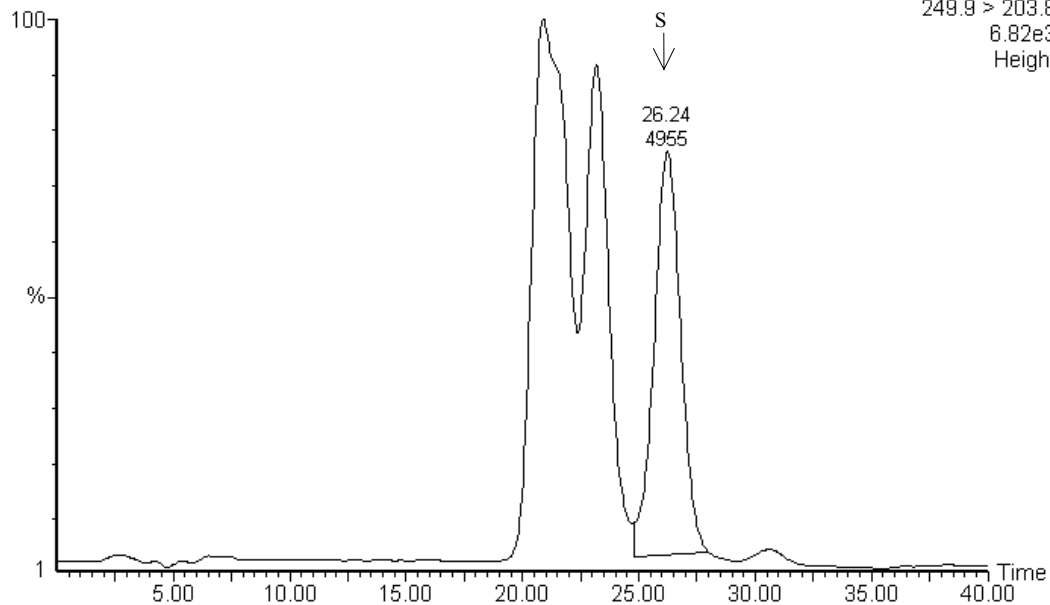
24-Sep-200201:28:16

MRM of 1 Channel ES+

249.9 > 203.8

6.82e3

Height



5. control + 0.05 ppm SYN508500, 8.83 mg injected, 0.415 ng found, 0.047 ppm, 93.9% recovery

0.05 PPM REC

47-TOM-04 Sm (Mn, 3x3)

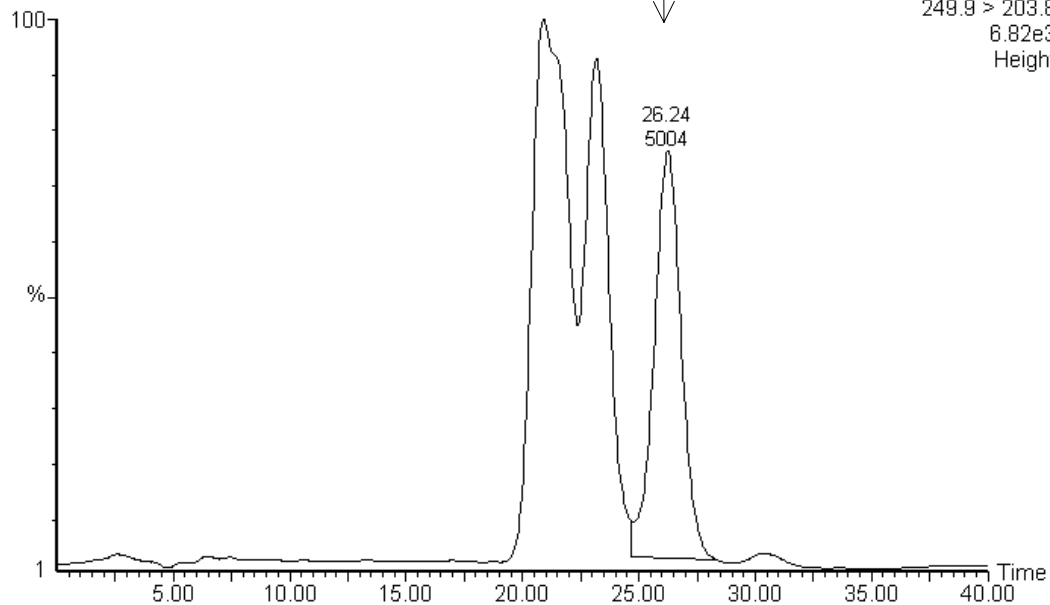
24-Sep-200202:09:26

MRM of 1 Channel ES+

249.9 > 203.8

6.82e3

Height



6. control + 0.05 ppm SYN508500, 8.83 mg injected, 0.419 ng found, 0.047 ppm, 94.9% recovery

FIGURE 10. CHROMATOGRAMS SYN508500 IN TOMATO (CONT.)

0.1 PPM REC

47-TOM-05 Sm (Mn, 3x3)

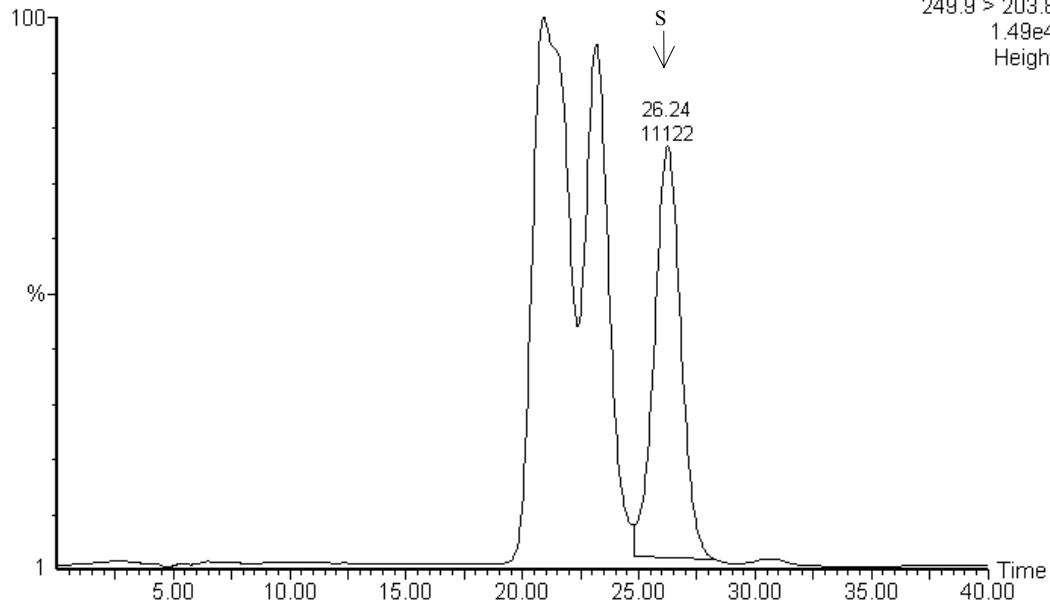
24-Sep-200202:50:23

MRM of 1 Channel ES+

249.9 > 203.8

1.49e4

Height



7. control + 0.10 ppm SYN508500, 8.83 mg injected, 0.979 ng found, 0.111 ppm, 111% recovery

0.1 PPM REC

47-TOM-06 Sm (Mn, 3x3)

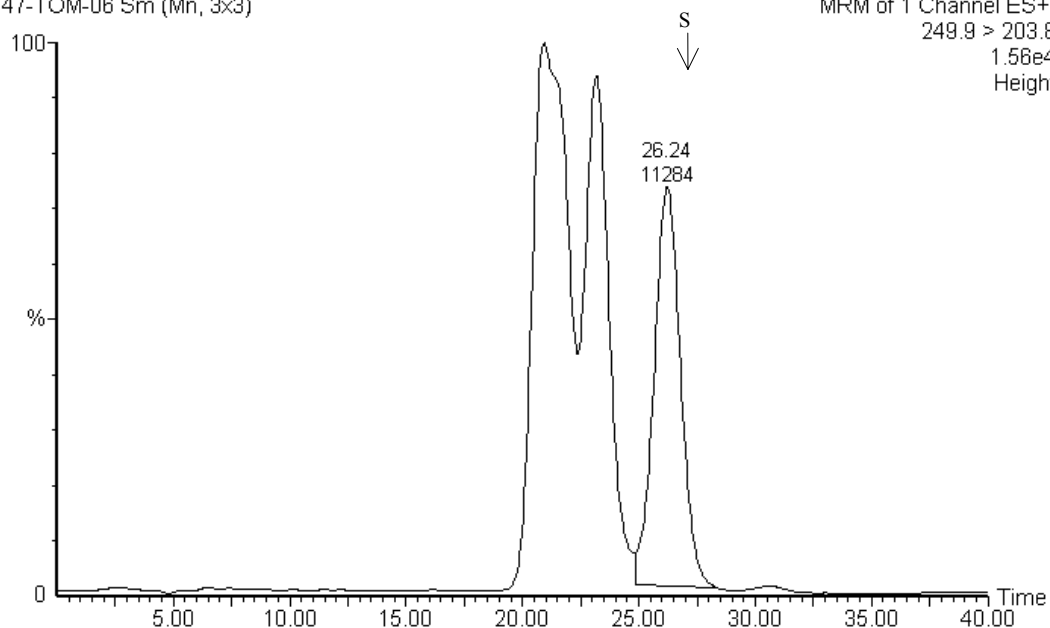
24-Sep-200203:31:18

MRM of 1 Channel ES+

249.9 > 203.8

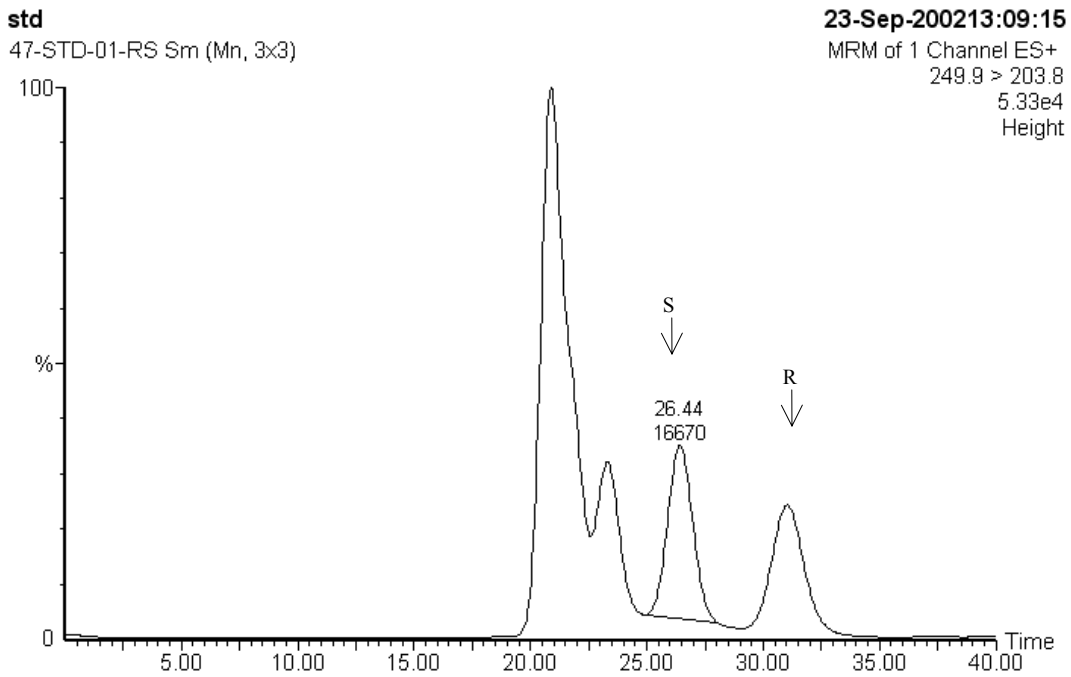
1.58e4

Height

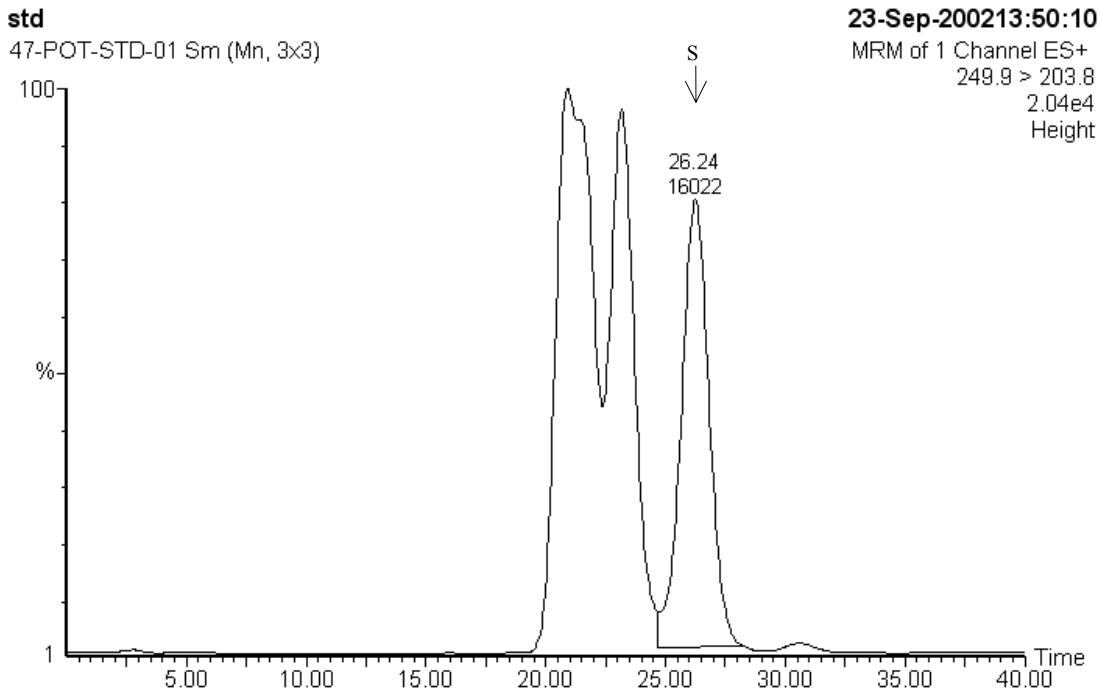


8. control + 0.10 ppm SYN508500, 8.83 mg injected, 0.994 ng found, 0.113 ppm, 113% recovery

FIGURE 11. CHROMATOGRAMS SYN508500 IN POTATO

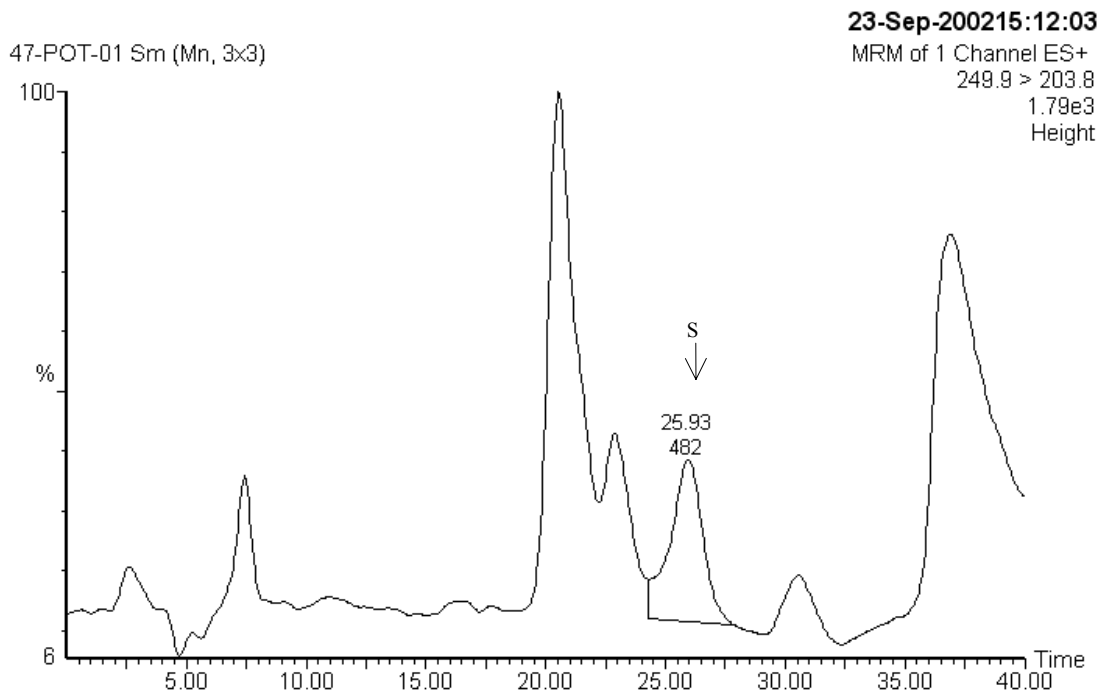


1. 1.25 ng standard, CGA-49751 (Racemic Standard)

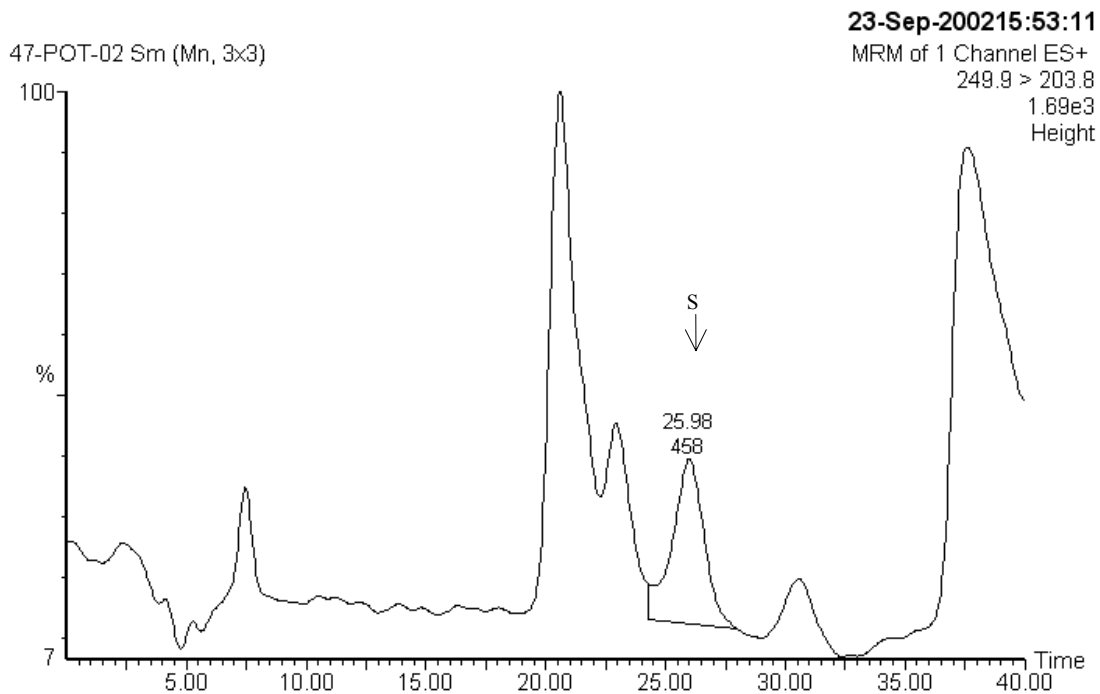


2. 1.25 ng standard, CGA-508500

FIGURE 11. CHROMATOGRAMS SYN508500 IN POTATO (CONT.)

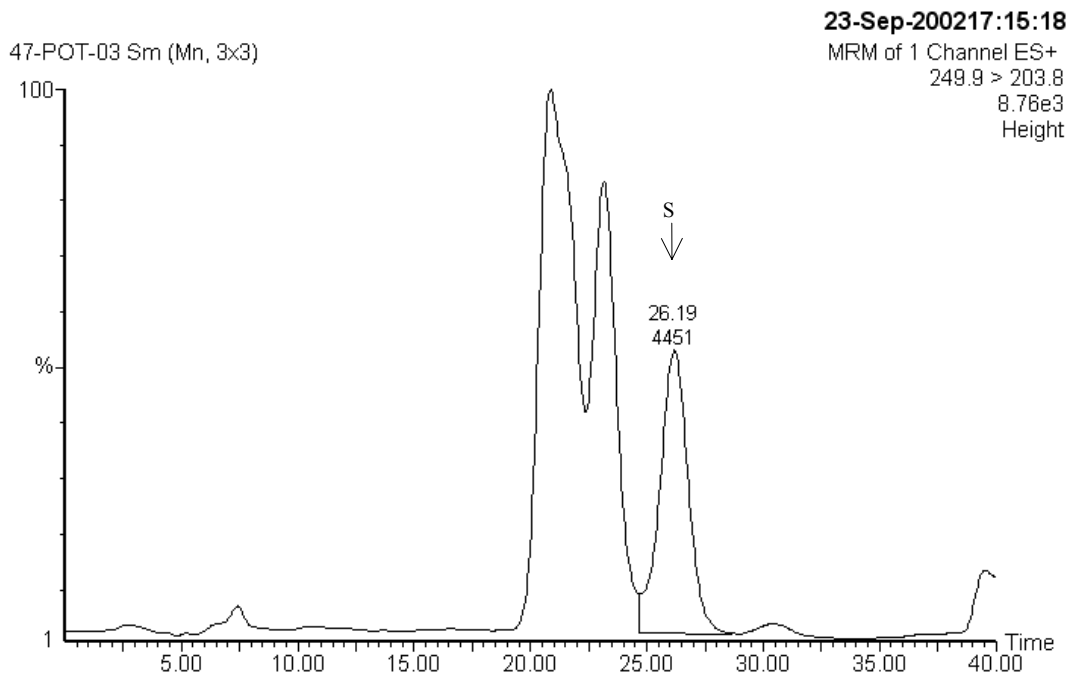


3. control, 8.84 mg injected, 0.00 ng found, <0.05 ppm

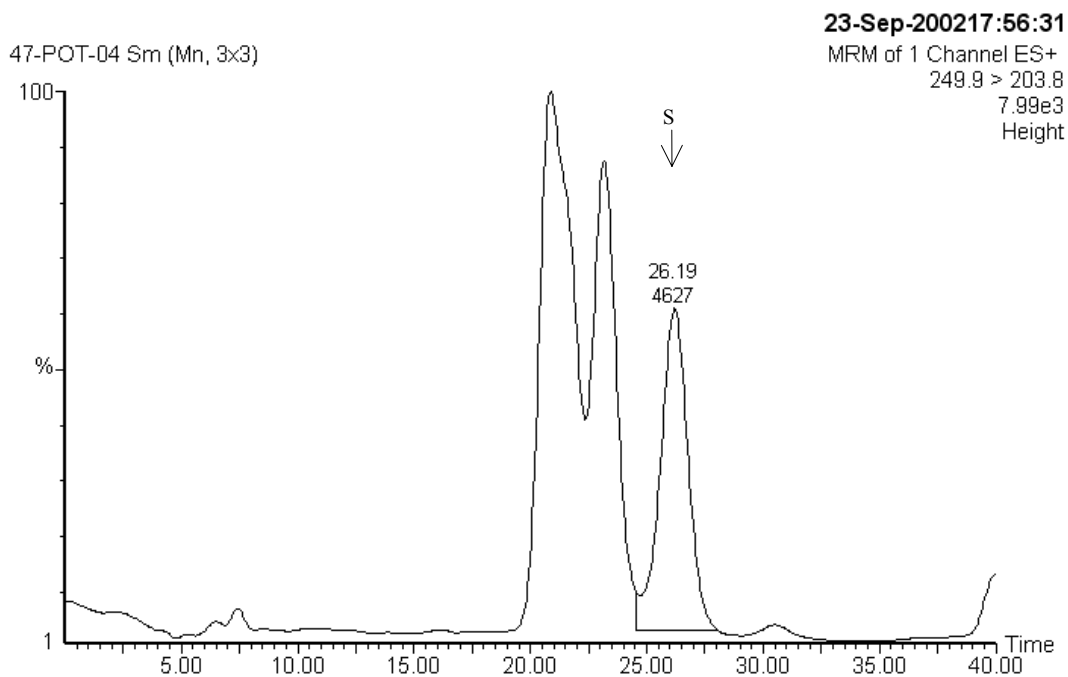


4. control, 8.84 mg injected, 0.00 ng found, <0.05 ppm

FIGURE 11. CHROMATOGRAMS SYN508500 IN POTATO (CONT.)

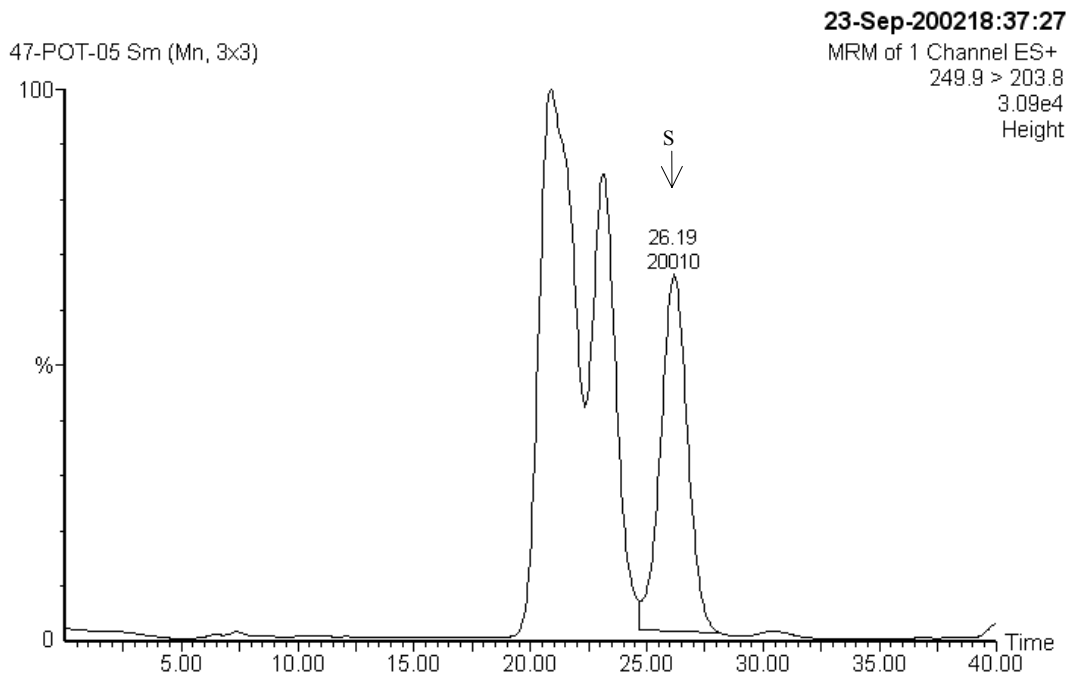


5. control + 0.05 ppm SYN508500, 8.84 mg injected, 0.321 ng found, 0.036 ppm, 72.7% recovery

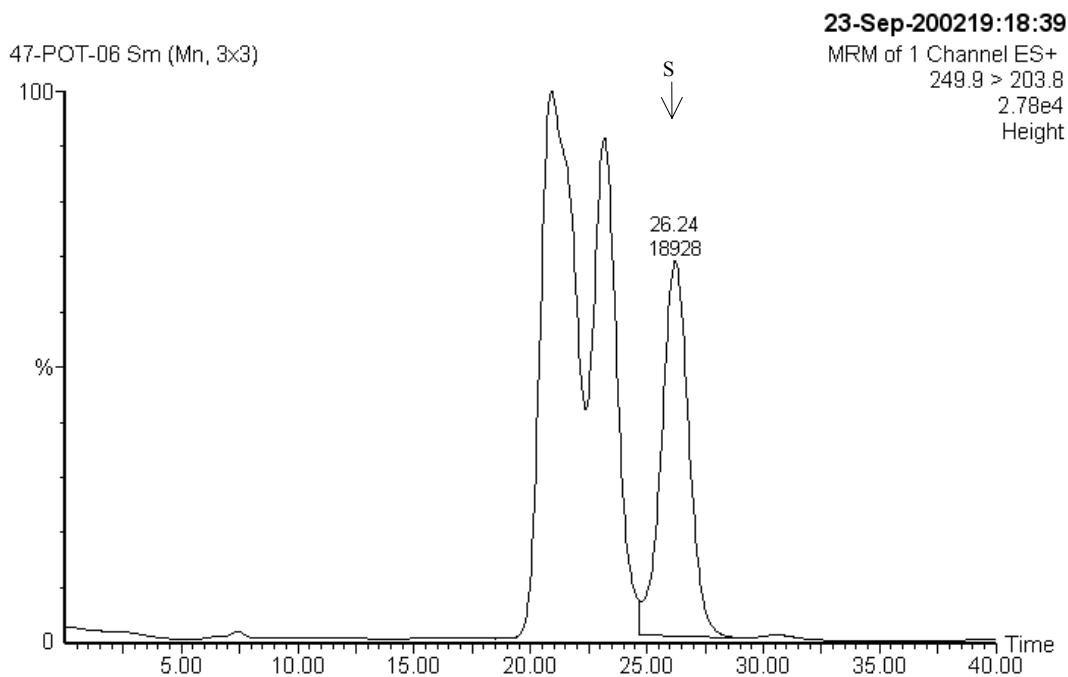


6. control + 0.05 ppm SYN508500, 8.84 mg injected, 0.336 ng found, 0.038 ppm, 76.1% recovery

FIGURE 11. CHROMATOGRAMS SYN508500 IN POTATO (CONT.)

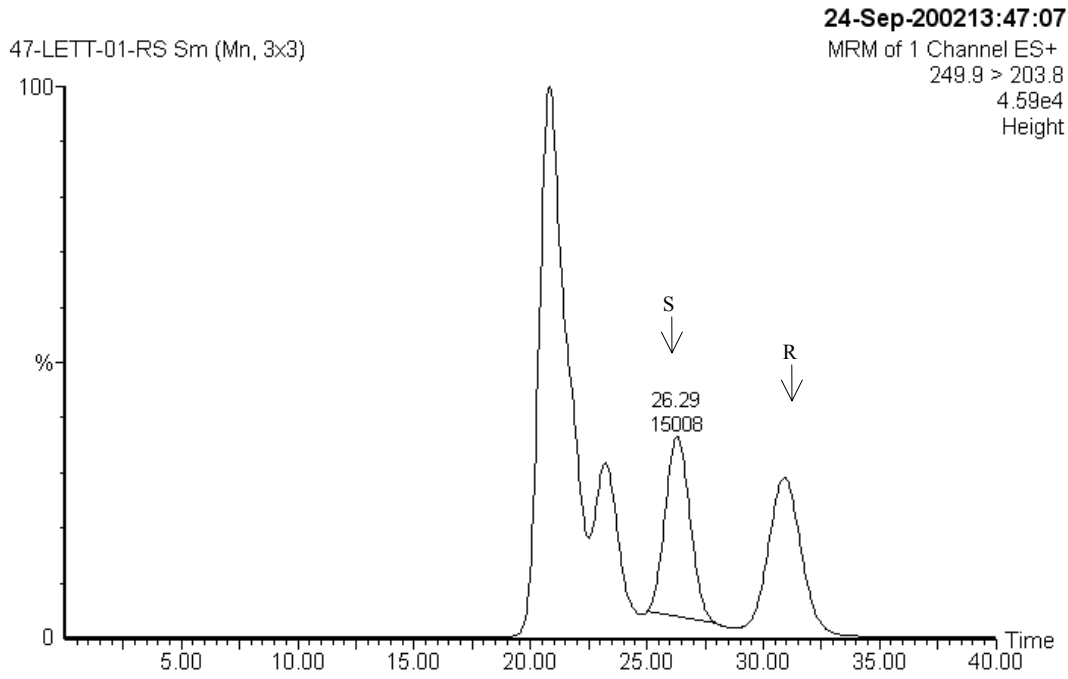


7. control + 0.20 ppm SYN508500, 8.84 mg injected, 1.66 ng found, 0.188 ppm, 93.9% recovery

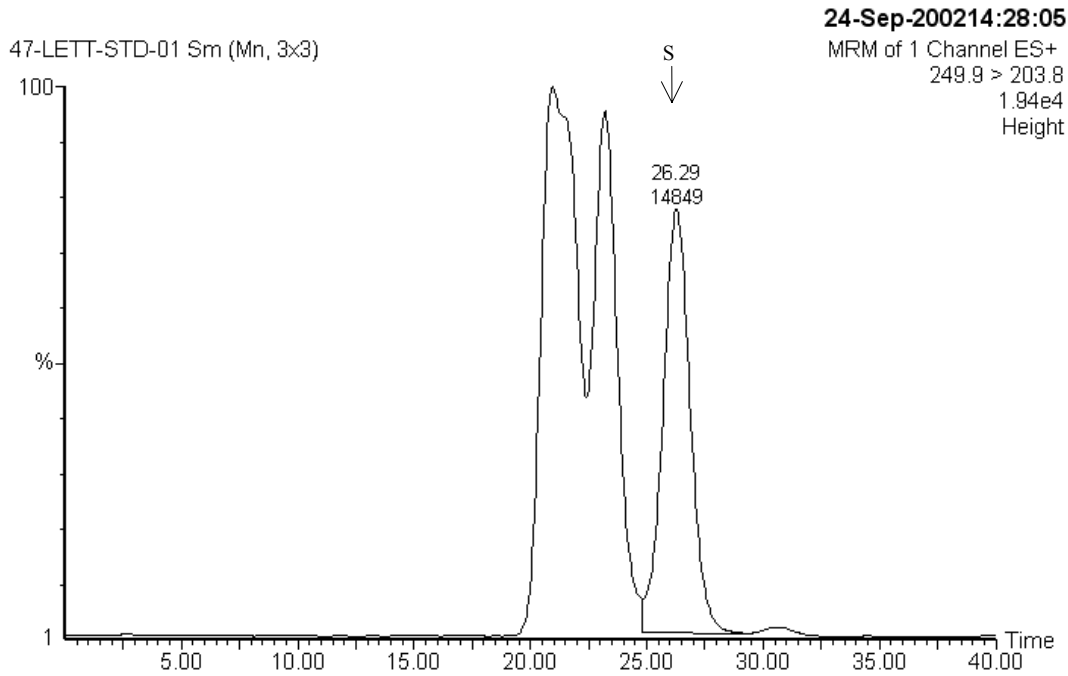


8. control + 0.20 ppm SYN508500, 8.84 mg injected, 1.57 ng found, 0.177 ppm, 88.7% recovery

FIGURE 12. CHROMATOGRAMS SYN508500 IN LEAF LETTUCE

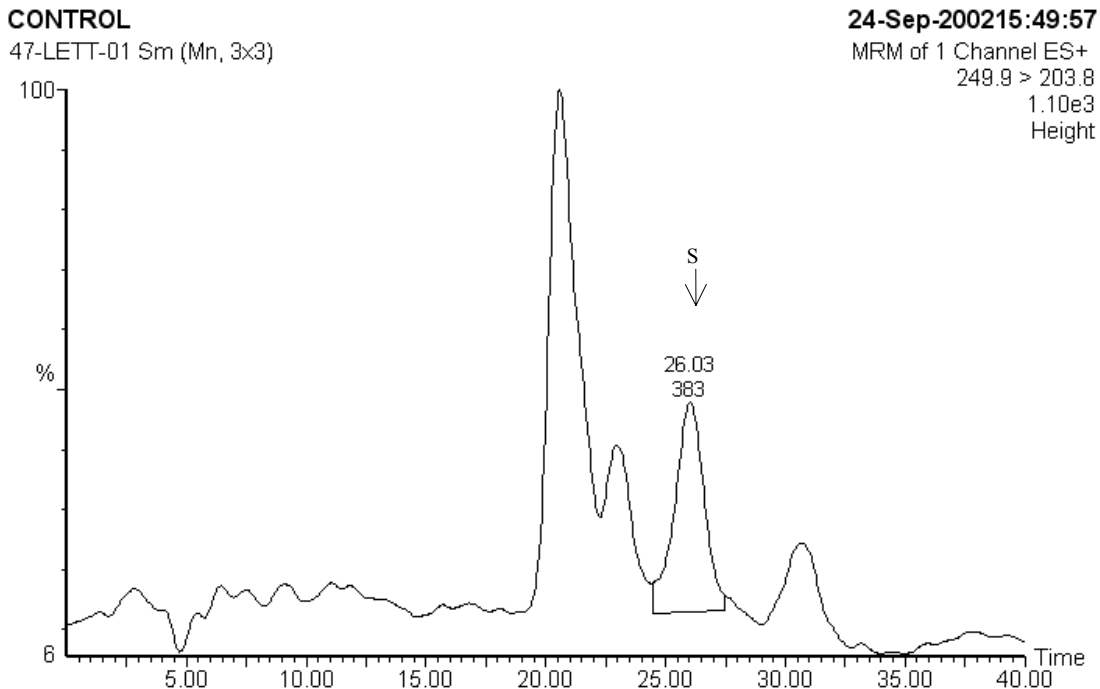


1. 1.25 ng standard, CGA-49751 (Racemic Standard)

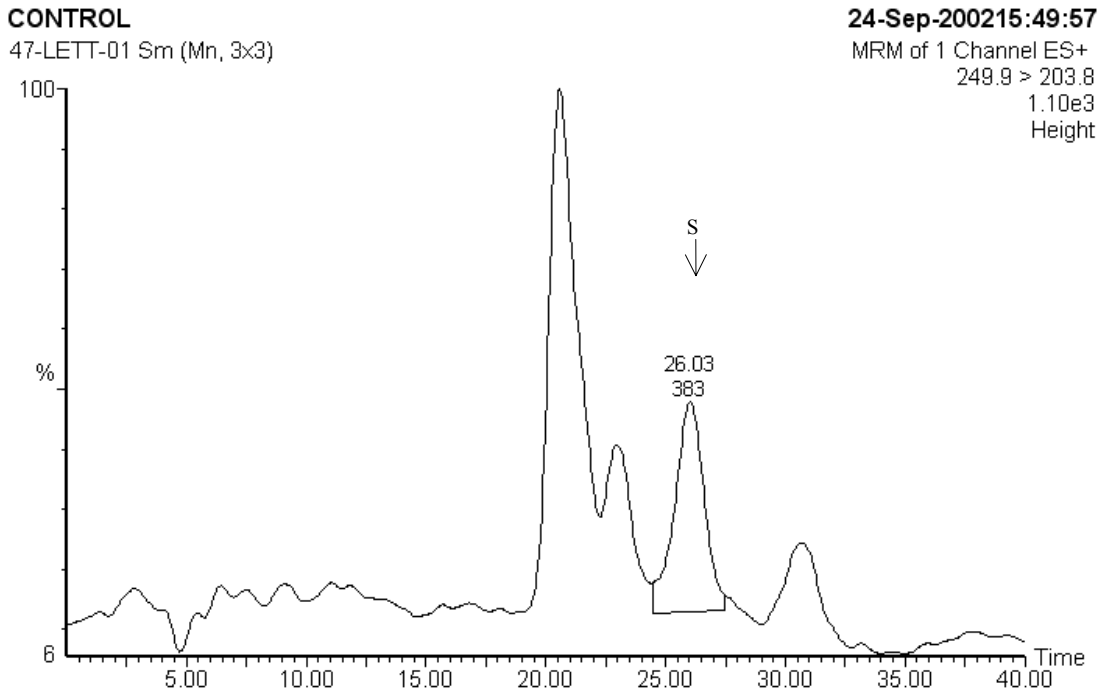


2. 1.25 ng standard, SYN508500

FIGURE 12. CHROMATOGRAMS SYN508500 IN LEAF LETTUCE (CONT.)



3. control, 8.74 mg injected, 0.00 ng found, <0.05 ppm



4. control, 8.74 mg injected, 0.00 ng found, <0.05 ppm

FIGURE 12. CHROMATOGRAMS SYN508500 IN LEAF LETTUCE (CONT.)

0.05 PPM REC

47-LETT-03 Sm (Mn, 3x3)

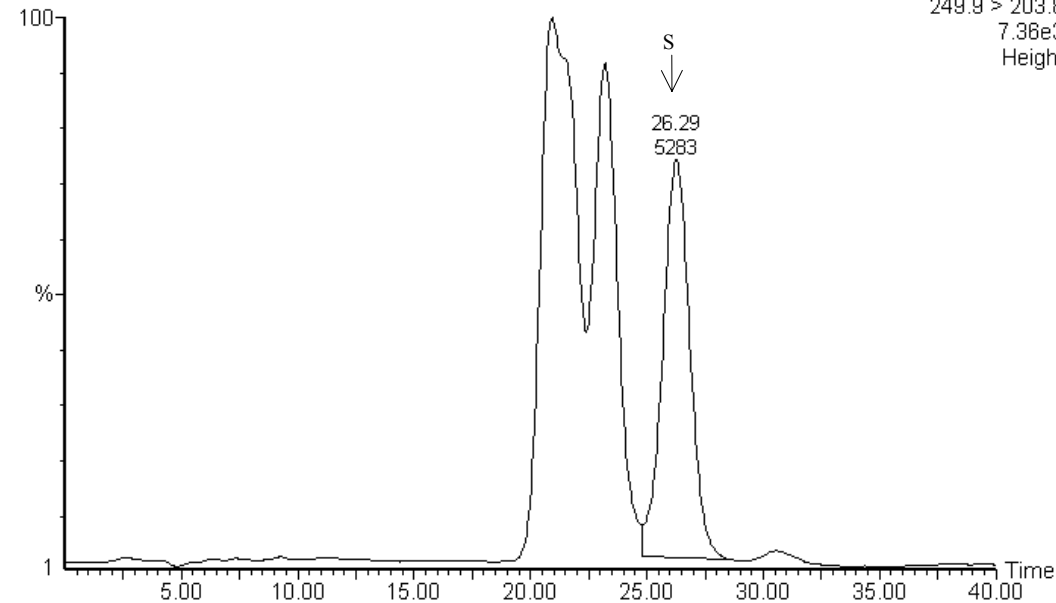
24-Sep-200217:53:17

MRM of 1 Channel ES+

249.9 > 203.8

7.36e3

Height



5. control + 0.05 ppm SYN508500, 8.74 mg injected, 0.427 ng found, 0.049 ppm, 97.7% recovery

0.05 PPM REC

47-LETT-04 Sm (Mn, 3x3)

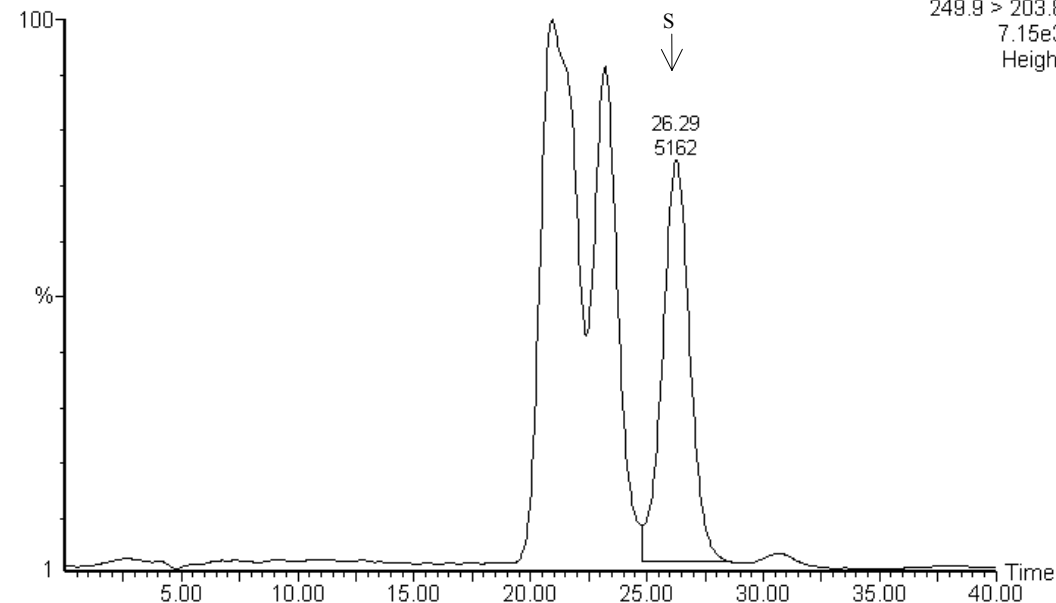
24-Sep-200218:34:32

MRM of 1 Channel ES+

249.9 > 203.8

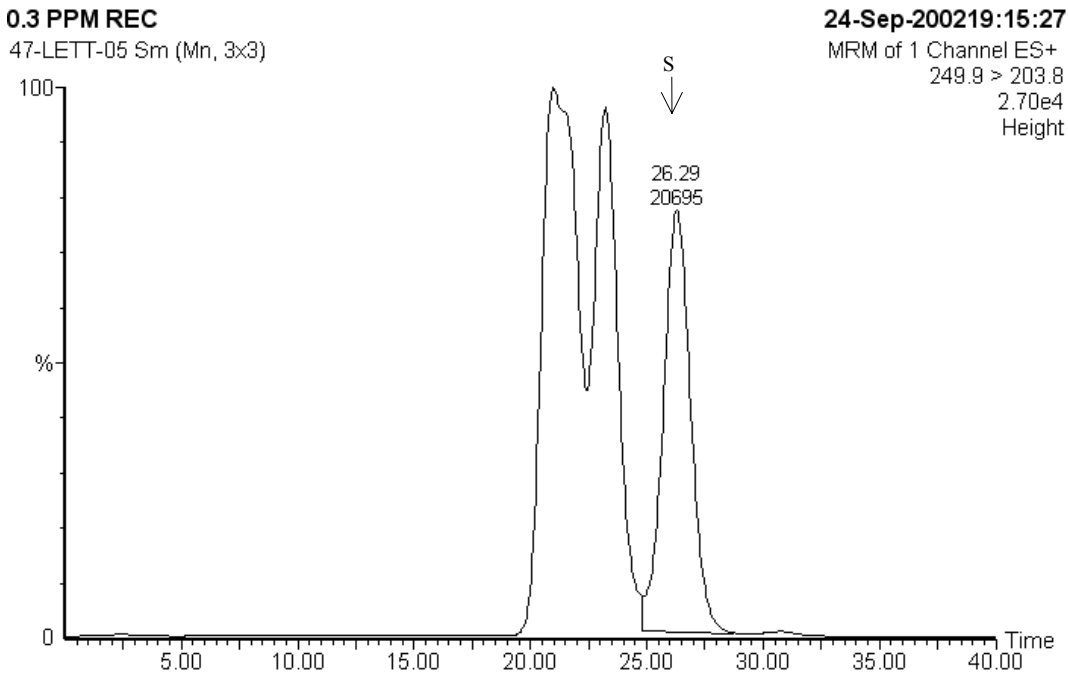
7.15e3

Height

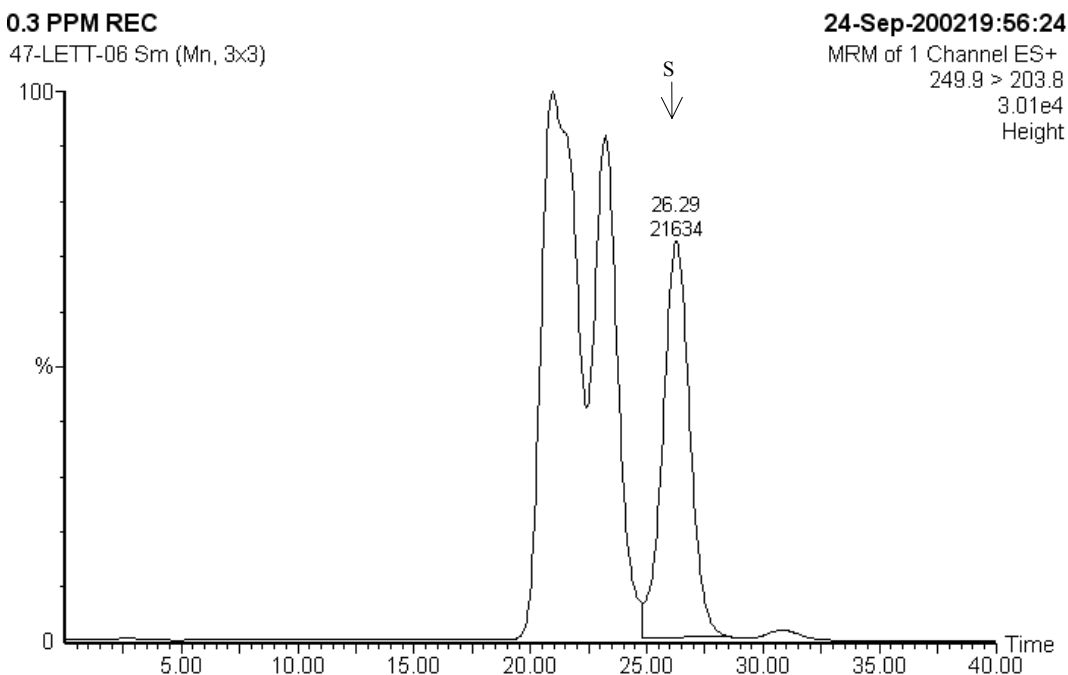


6. control + 0.05 ppm SYN508500, 8.74 mg injected, 0.416 ng found, 0.048 ppm, 95.2% recovery

FIGURE 12. CHROMATOGRAMS SYN508500 IN LEAF LETTUCE (CONT.)

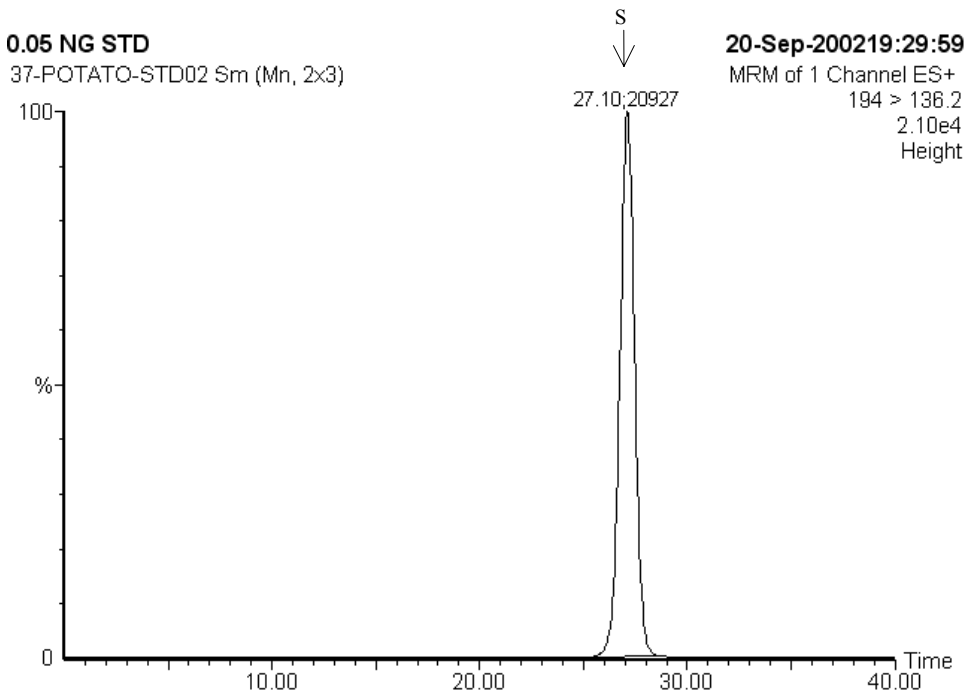


7. control + 0.30 ppm SYN508500, 5.83 mg injected, 1.86 ng found, 0.319 ppm, 106% recovery

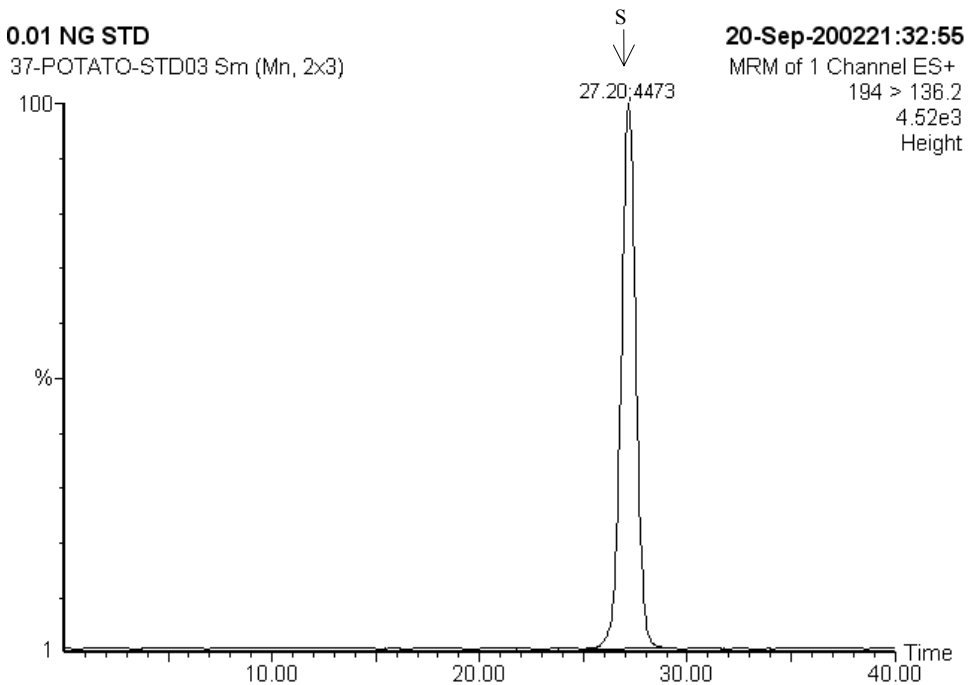


8. control + 0.30 ppm SYN508500, 5.83 mg injected, 1.94 ng found, 0.334 ppm, 111% recovery

FIGURE 13. CALIBRATION STANDARDS SYN506357

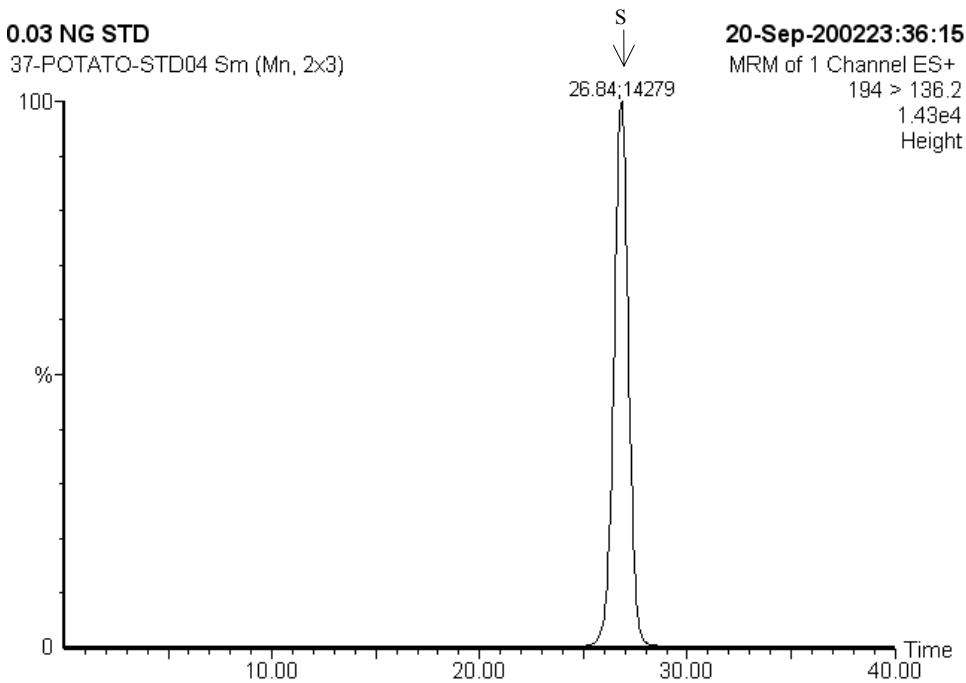


1. 1.25 ng standard SYN506357, Response Factor = 20927

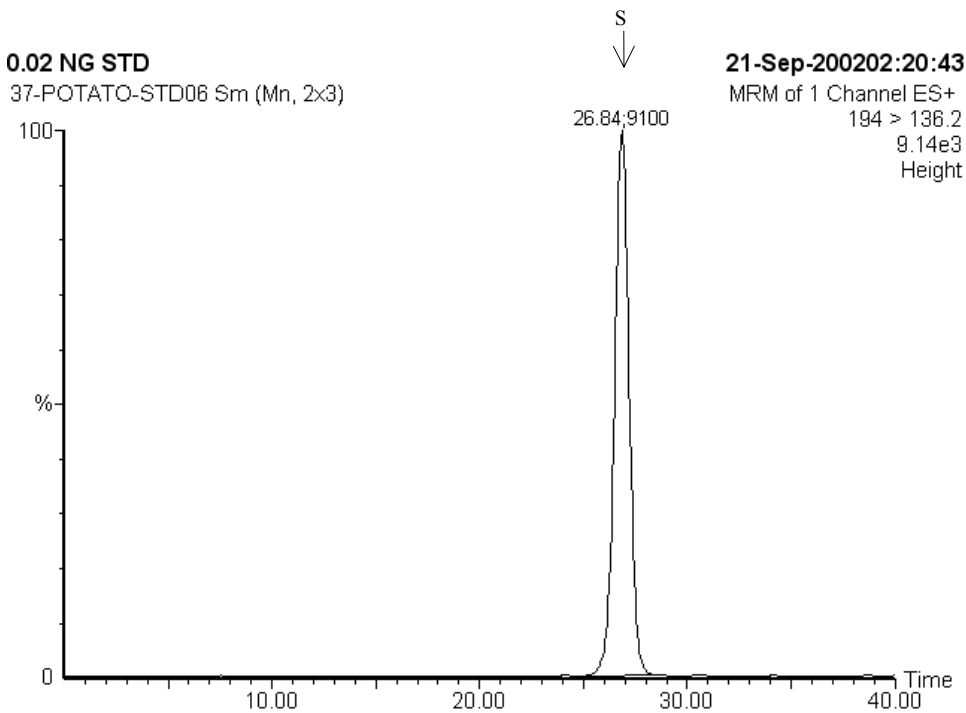


2. 0.25 ng standard SYN506357, Response Factor = 4473

FIGURE 13. CALIBRATION STANDARDS SYN506357 (CONT.)

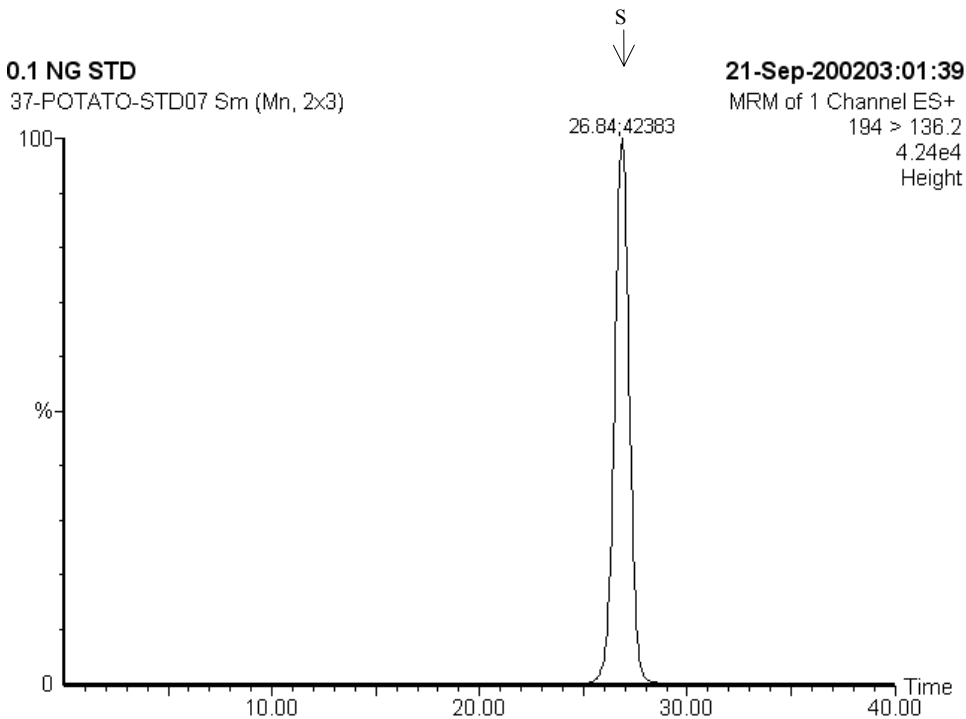


3. 0.75 ng standard SYN506357, Response Factor = 14279

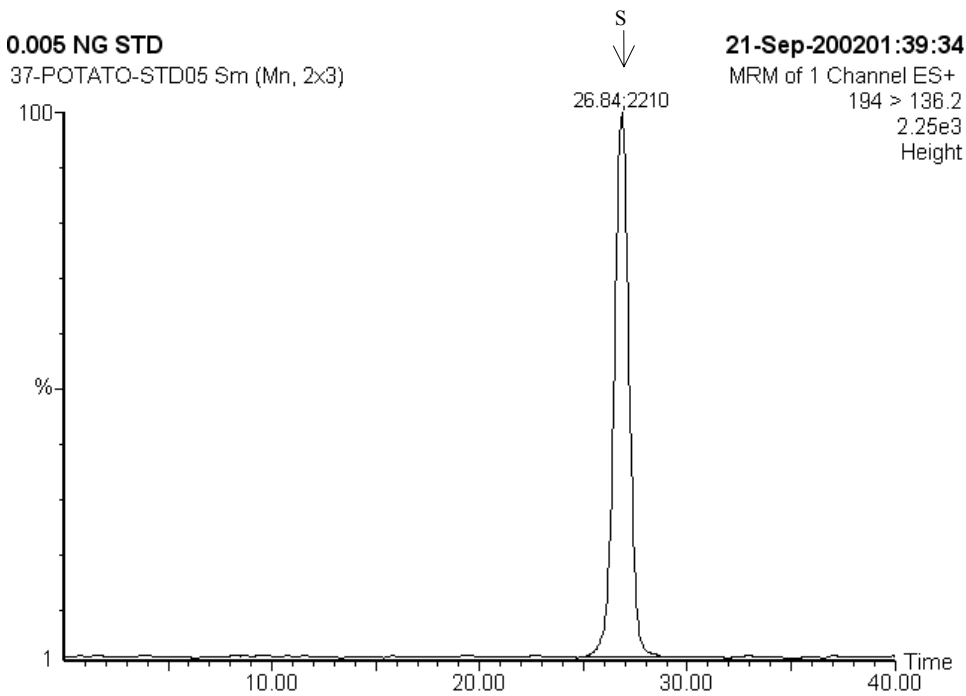


4. 0.50 ng standard SYN506357, Response Factor = 9100

FIGURE 13. CALIBRATION STANDARDS SYN506357 (CONT.)



5. 2.50 ng standard SYN506357, Response Factor = 42392



6. 0.125 ng standard SYN506357, Response Factor = 2210

FIGURE 14. TYPICAL CALIBRATION CURVE SYN506357

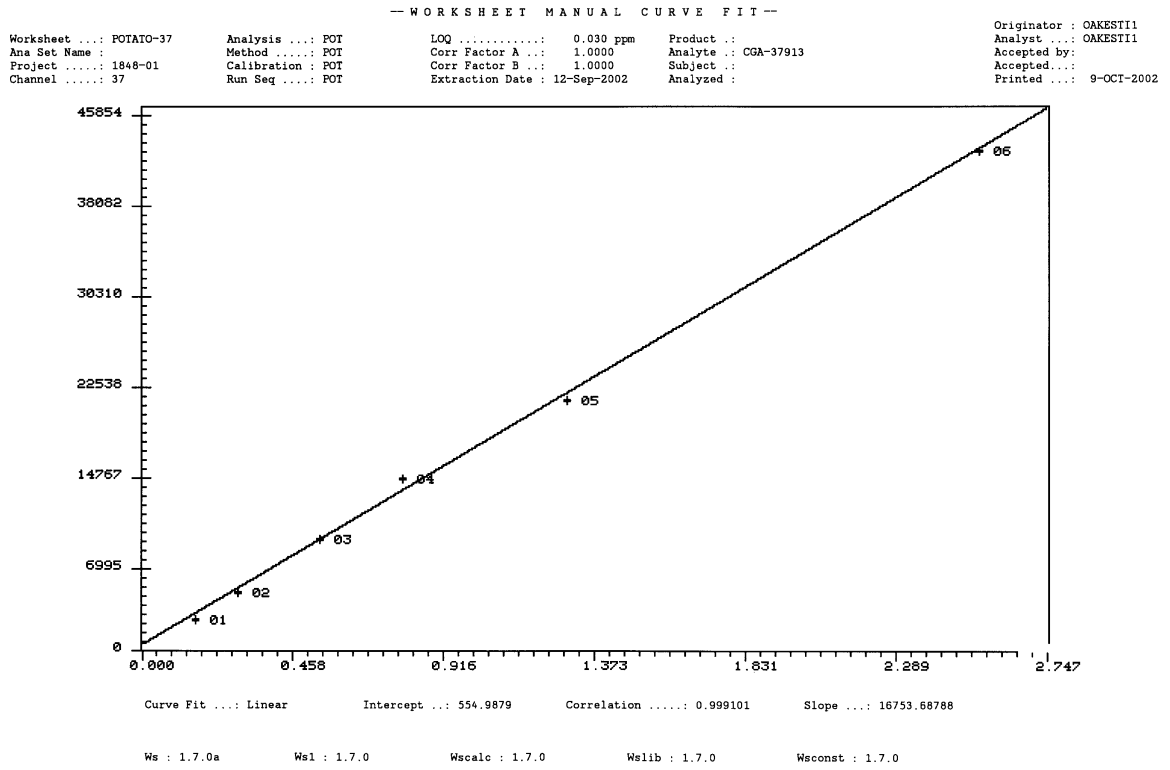
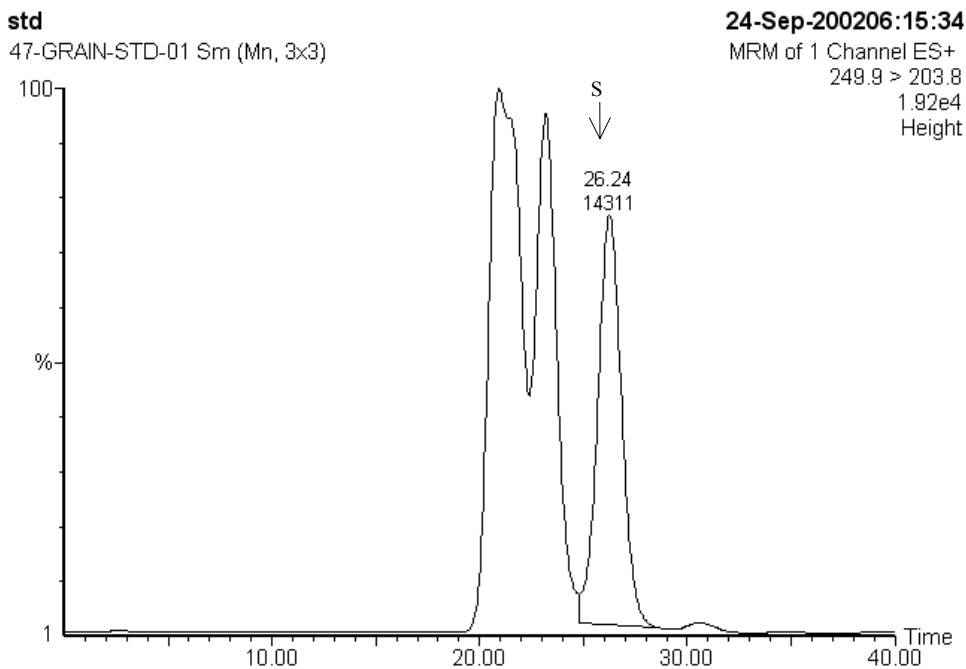
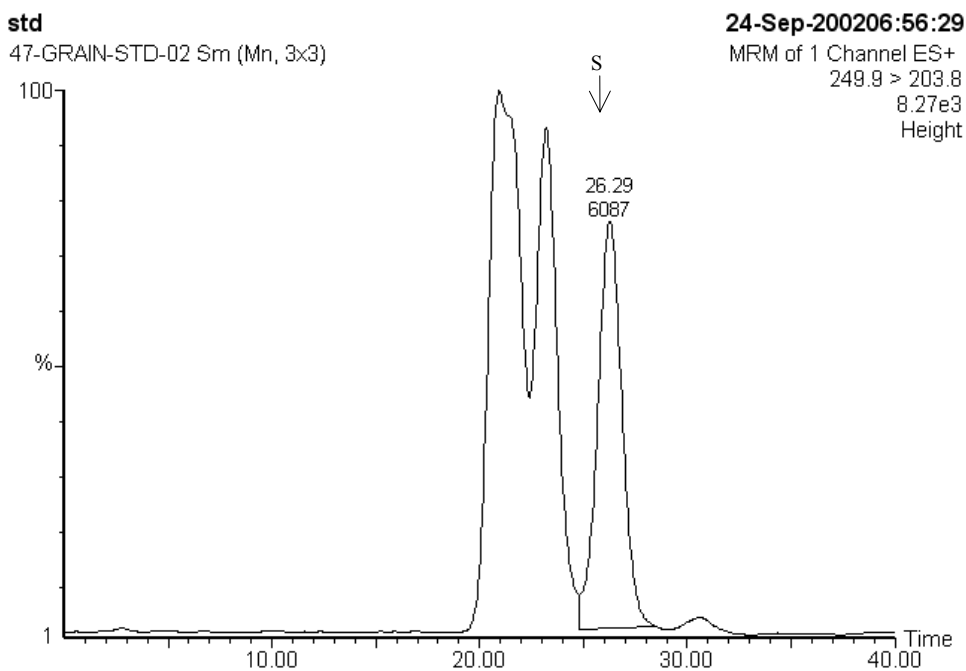


FIGURE 15. CALIBRATION STANDARDS SYN508500

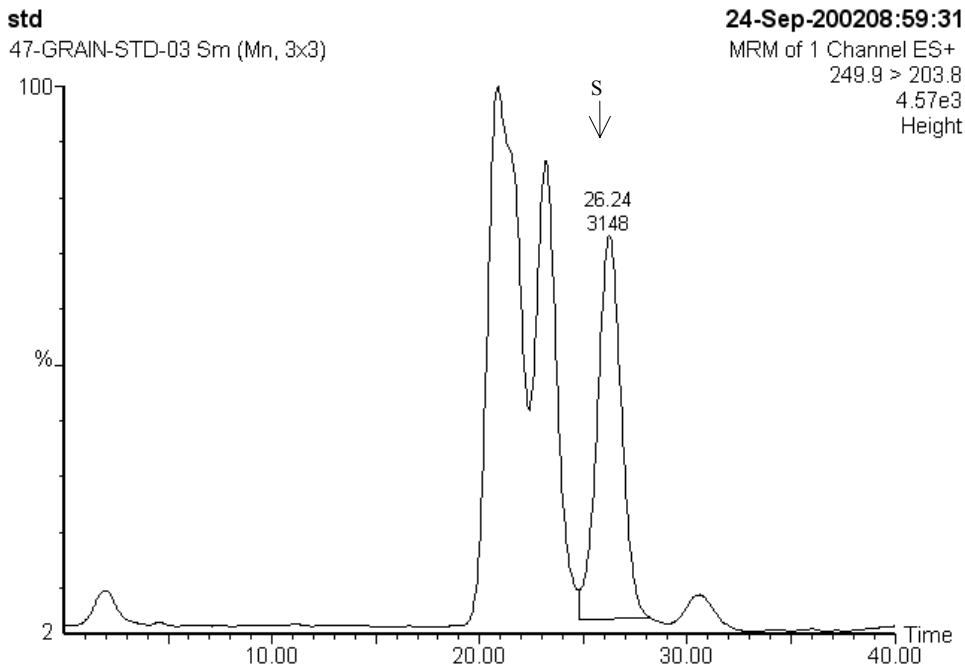


1. 1.25 ng standard SYN508500, Response Factor = 14311

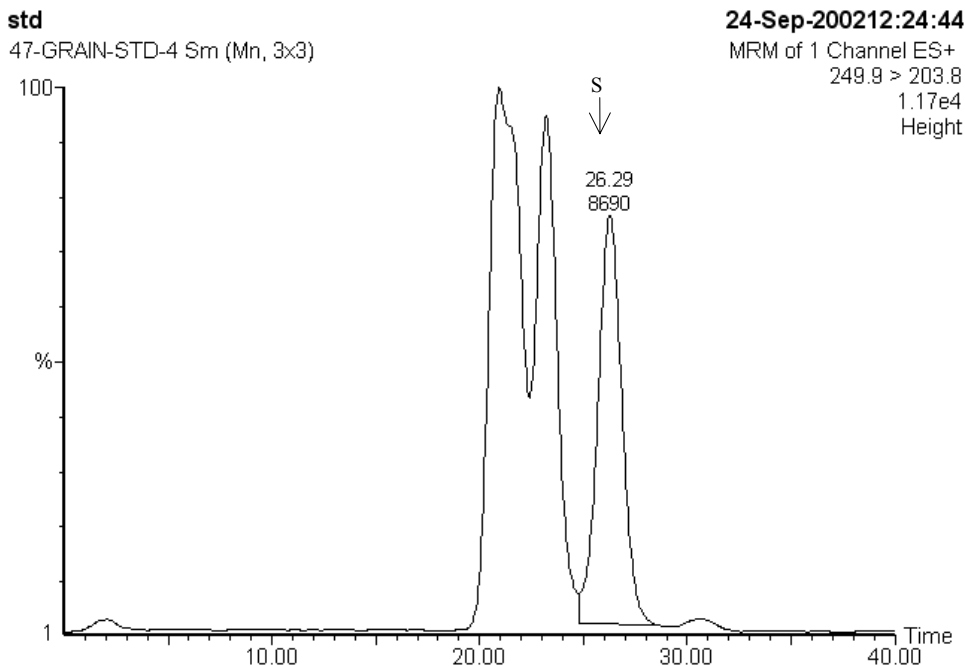


2. 0.5 ng standard SYN508500, Response Factor = 6087

FIGURE 15. CALIBRATION STANDARDS SYN508500 (CONT.)

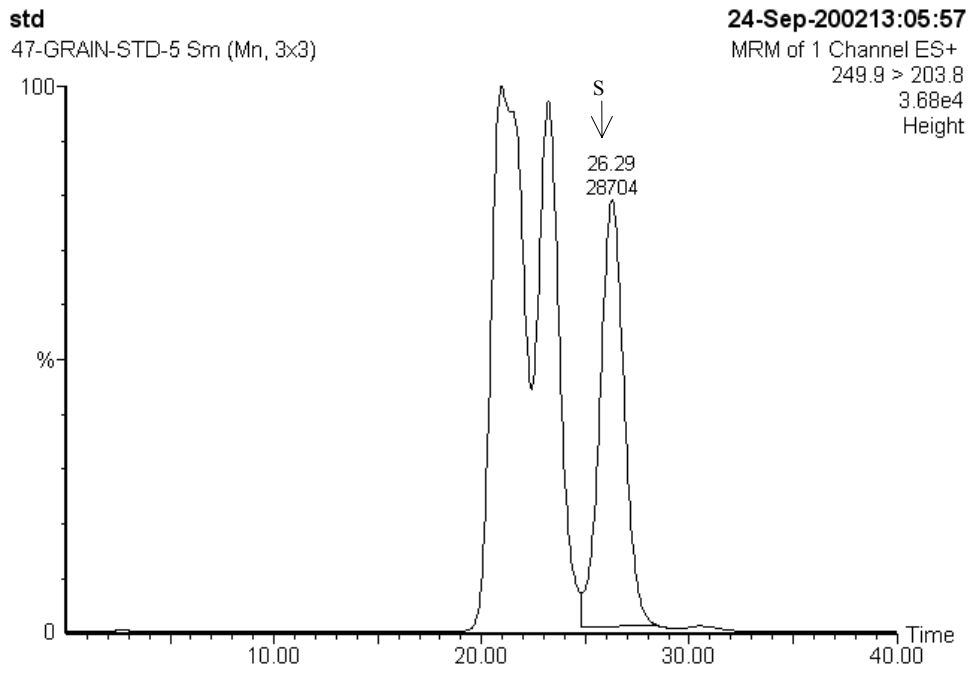


3. 0.25 ng standard SYN508500, Response Factor = 3148



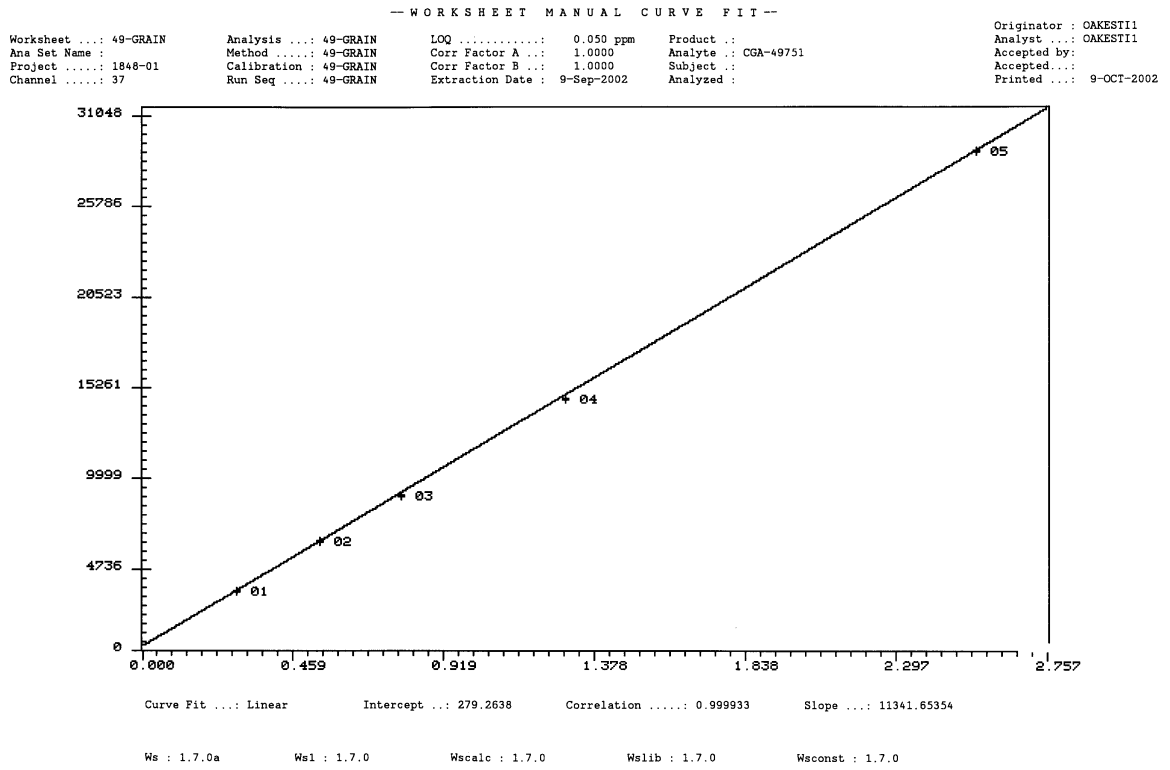
4. 0.75 ng standard SYN508500, Response Factor = 8690

FIGURE 15. CALIBRATION STANDARDS SYN508500 (CONT.)



5. 2.5 ng standard SYN508500, Response Factor = 28704

FIGURE 16. TYPICAL CALIBRATION CURVE, SYN508500



6.0 REFERENCES

1. Cargile, N. L., and Ross, J. A., AG-338, “Analytical Method for Residues of Metolachlor Plant Metabolites Determined as CGA-37913 and CGA-49751 after Acid Hydrolysis. MRID 00015543.
2. Wurz, R. E. M., Grunenwald, M. C. and Van Geluwe, K., AG-612, “Analytical Method For The Determination Of Metolachlor As CGA-37913 And CGA-49751 In Crops By Capillary Gas Chromatography.” MRID 43661202.
3. EN-CAS Method No. ENC-5/99, “Analytical Method for the Determination of Metolachlor Residues, as Metabolites CGA-37913 and CGA-49751, in Agricultural Commodities.” APPENDIX 2 to MRID 4555901.

7.0 APPENDIX

APPENDIX 1. SEPARATE VOLUMES PREVIOUSLY SUBMITTED

1. Cargile, N. L., and Ross, J. A., AG-338, “Analytical Method for Residues of Metolachlor Plant Metabolites Determined as CGA-37913 and CGA-49751 after Acid Hydrolysis. MRID 00015543.
2. Wurz, R. E. M., Grunenwald, M. C. and Van Geluwe, K., AG-612, “Analytical Method For The Determination Of Metolachlor As CGA-37913 And CGA-49751 In Crops By Capillary Gas Chromatography.” MRID 43661202.
3. EN-CAS Method No. ENC-5/99, “Analytical Method for the Determination of Metolachlor Residues, as Metabolites CGA-37913 and CGA-49751, in Agricultural Commodities.” APPENDIX 2 to MRID 45555901.