Cover Sheet for

Environmental Chemistry Method

Pesticide Name: Trifloxystrobin

MRID#: 453718-31

Matrix: Soil

Analysis: LC/MS/MS

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VOLUME ____ OF ____ OF SUBMISSION

CGA-362622: Method Amendment

TITLE

Analytical Method For The Determination Of CGA-362622 And Its Degradates CGA-53052, CGA-368732, CGA-368733, NOA-436664, AND CGA-382997 In Soil By High Performance Liquid Chromatography With Mass Spectrometric Detection Including Validation Data

DATA REQUIREMENT

EPA Guideline No. 164-1

AUTHOR

John D. Vargo, Ph.D.

COMPLETION DATE

February 10, 2000

PERFORMING LABORATORY

Environmental Safety Department
Novartis Crop Protection, Inc.
Greensboro, NC

LABORATORY STUDY IDENTIFICATION

Novartis Number 53-98
Analytical Method Number AG-692

SUBMITTER/SPONSOR

Novartis Crop Protection, Inc.
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VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 102

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B) or (C).

Company: Novartis Crop Protection, Inc.

Company Representative: Thomas J. Parshley

Title: Senior Regulatory Manager

Signature: Thomas J Parshley Date: 2-3-00

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Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other provision of common law or statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The analytical work reported in Study 53-98 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

John D. Vargo

2-10-2000

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REPORT APPROVAL

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QUALITY ASSURANCE STATEMENT

Study Title: Analytical Method for the Determination of CGA-362622 and Its Degradates CGA-53052, CGA-368732, CGA-368733, NOA-436664 and CGA-382997 in Soil by High Performance Liquid Chromatography with Mass Spectrometric Detection, Including Validation Data

Study Director: John D. Vargo

Study Number: 53-98

Pursuant to Good Laboratory Practice Regulations, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Date
Audit Protocol	12/03/98 - 12/03/98	12/03/98
Inspect Analytical	02/10/99 - 02/10/99	02/10/99
Audit Final Report	05/04/99 - 05/06/99	05/10/99
Inspect Analytical	12/15/99 - 12/15/99	12/15/99
Audit Final Report Amendment	01/31/00 - 02/02/00	02/02/00

Prepared by: Teresa S Cox Date: 2/2/00

Teresa S Cox
Staff Quality Assurance Auditor
Quality Assurance Unit
Novartis Crop Protection, Inc.

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GENERAL INFORMATION

Study Participants

John D. Vargo, Scientist III
Study Director, Sample Preparation/Analysis/Data Analysis/Final Report

Ben Merritt, Lab Technician
Sample Preparation

Terry Blackburn, Sample Coordinator
Sample Custody/Sample Homogenation

Study Initiation Date: January 29, 1999

Study Reopen Date: December 1, 1999

Circumstances Affecting Quality or Integrity of Data

A total of three sets of 0.5 ppb level fortifications for the 0-6" soil layer were prepared and analyzed due to marginal recoveries for CGA-382997 in sample sets. Enhanced recoveries (163% for sample LA0-6-0.5-10 and 407% for sample LA0-6-0.5-15) were observed in 2 of 15 samples for 0-6" soil fortified at 0.5 ppb. The enhanced recoveries are not typical and the cause is unknown. These two samples were omitted from statistical calculations for analyte CGA-368733.

Reference Substances

Analytical Standard	Novartis Inventory Number	% Purity	Expiration/ Re-analysis Date
CGA-362622	S97-2126	95.6	10/2000
CGA-53052	NEH-XVI-52	99.5	5/2001
CGA-382997	NEH-XVI-51-5	99.8	5/2001
CGA-368732	NEH-XVII-14	99.7	8/2001
CGA-368733	NEH-XVII-16	98.7	9/2001
NOA-436664	JWP-IV-85-4	99.8	8/2000

Soil Samples

The soil samples were collected as part of the CGA-362622 field dissipation study being conducted in Louisiana. Novartis study: 42-99. Project: H362622GBL000A. Sample inventory number: INV18460.2. Novartis test number: RIEN00699. These soil samples were collected on April 14, 1999, prior to the first application of the active ingredient. The samples were homogenized at Novartis Crop Protection, Greensboro, NC. The samples were stored frozen since the time of collection.

0-6" soil zone. Novartis sample id: 270288
6-12" soil zone. Novartis sample id: 270313
12-18" soil zone. Novartis sample id: 270290

I. INTRODUCTION/SUMMARY

A. Scope

This method is used for the determination of CGA-362622 (Chemical Abstracts Registry (CAS) Number: 199119-58-9) and its degradates CGA-53052, CGA-368732, CGA-368733, NOA-436664, and CGA-382997 in soil. The compounds are separated by high performance liquid chromatography (HPLC) and detected by mass spectrometry (LC/MS/MS). The limit of detection by LC/MS/MS (smallest standard amount injected during the chromatographic run) is 0.0125 ng injected for all analytes. The limit of quantification (LOQ) (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for LC/MS/MS analyses is 0.5 ppb for all analytes in soil.

The results presented in this method amendment are for revalidation experiments of analytical method AG-692[1] with some modifications made to the extraction procedure. Low recoveries of analytes CGA-368733 and NOA-436664 were observed using method AG-692 as written when analyzing samples from a terrestrial field dissipation study being conducted in Louisiana. Subsequent method development experiments indicated a need to perform a more rigorous extraction. The experiments also indicated that the 0-6" soil layer needed a slightly different extraction procedure than soils of depths greater than 6".

The only modifications made to AG-692 involve the nature and sequence of the extraction solvents. All other aspects of the subsequent sample cleanup remain the same. The HPLC gradient has been slightly modified to lessen the analysis time. All changes will be noted in this method amendment. Sections that are unchanged from AG-692 will only reference the original method. All data and chromatograms from these additional validation studies will be presented.

B. Principle

A 20-gram subsample of soil is extracted three times with a series of extraction solvents (once with 70% (v/v) methanol/water and twice with 70% (v/v) methanol/water, 2% in ammonium hydroxide for 0-6" soil layers, and three times with 70% (v/v) methanol/water, 2% in ammonium hydroxide for soils of depths greater than 6") at room temperature using mechanical agitation. The sample is centrifuged and filtered with extracts combined. The methanol content is removed by rotary evaporation. The aqueous is passed through a SAX SPE column

attached piggy-back style to a ENV SPE column. The SAX SPE column, which retains soil matrix and does not retain the analytes, is detached and discarded. The analytes are eluted from the ENV SPE using acetonitrile. The samples are adjusted to the desired final volume and organic content. LC/MS/MS is used for analysis of the samples.

II. MATERIALS AND METHODS

A. Apparatus

The apparatus used in these additional method validation studies are the same as used and described in analytical method AG-692.

B. Reagents and Analytical Standards

The reagents and analytical standards used in these additional method validation studies are the same as used and described in analytical method AG-692.

C. Safety and Health

Whereas most of the chemicals used and analyzed for in this method have not been completely characterized, general laboratory safety is advised (e.g., safety glasses, gloves, etc. should be used). Acetic acid is an irritant and should be used in a well-ventilated area (i.e., a fume hood).

D. Analytical Procedure

Note: All glassware, including the polyallomer bottles used for soil extraction should be thoroughly cleaned and rinsed with acetonitrile or methanol prior to use. The analysis system is very sensitive and may detect contamination from previous samples if all glassware and extraction bottles are not properly cleaned prior to each use.

1.0 Soil Moisture Determination

The procedure used for soil moisture determination in these additional method validation studies is the same as used and described in analytical method AG-692.

2.0 Soil

Modifications made to the soil extraction/cleanup procedure are indicated in bold type.

Soil characterization data for the soils used in these experiments are presented in Table 1.

(Note: Samples must be homogenized prior to analysis using suitable sample preparation techniques.)

2.1 Weigh and record 20 ± 0.1 g of soil sample into a plastic extraction bottle.

2.2 Sample fortification, if required for this particular sample, is to be done at this time (refer to Section II.K.2.0).

2.3 Add 75 mL of the soil extraction solvent **(70% methanol/water for 0-6" soil samples, 70% methanol/water, 2% in ammonium hydroxide for soil depths >6")** to the sample.

2.4 **Shake the bottle vigorously for 5-10 seconds to break up soil clumps.** Place the sample on an orbital shaker and agitate the sample at high speed for thirty minutes at room temperature.

2.5 Centrifuge the sample at approximately 9,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.6 Decant the sample extract through filter paper into a 500-mL round bottom flask.

2.7 Repeat the extraction adding 75 mL of extraction solvent **(70% methanol/water, 2% in ammonium hydroxide)** to the sample and repeat Steps 2.4 through 2.6, adding the second extract to the first contained in the round bottom flask from Step 2.6.

2.8 Repeat the extraction adding 75 mL of extraction solvent (70% methanol/water, 2% in ammonium hydroxide) to the sample and repeat Steps 2.4

through 2.6, adding the third extract to the round bottom flask from Step 2.6. When all of the extract has passed through the filter paper, rinse the filter paper with approximately 10 mL of methanol, using a squirt bottle, prior to initiating the rotary evaporation step.

2.9 Remove methanol from the sample until only aqueous remains via rotary evaporation with a water bath temperature of approximately 35 °C. Add water, if necessary, to prevent the sample from going dry. There should be a minimum aqueous volume of approximately 25 mL.

2.10 **Add 10 mL of 0.1 M ammonium carbonate to basify the sample.** If the sample is cloudy with suspended particulates, transfer the sample to a centrifugable plastic bottle, using several mL of 0.1% ammonium carbonate to rinse the round bottom flask, adding this rinsate to the bottle. Centrifuge the sample at approximately 9,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.11 Pass the sample through a Varian SAX SPE column attached piggy-back style to a Varian ENV SPE. Discard the eluate. (The SPE columns are preconditioned by passing 10 mL of methanol and then 10 mL of 0.1 M ammonium carbonate through each column. Add 2-3 mL of 0.1 M ammonium carbonate to the lower ENV column just prior to adding the sample to the top SAX column. Do not permit the lower ENV column to go dry while the top SAX column still contains sample. Add 0.1 M ammonium carbonate to the lower ENV column, as needed, to prevent it from going dry.)

2.12 Add approximately 10 mL of 0.1 M ammonium carbonate to the round bottom flask from Step 2.9 and swirl to dissolve any residues still on the glass. Pass through both SPE columns. Discard the eluate. Pass an additional 3-4 mL of 0.1 M ammonium carbonate through both SPE columns. Discard the eluate.

2.13 Disconnect the SAX column and discard. Rinse the ENV column with approximately 3 mL of 0.1 M ammonium carbonate, followed by 6 mL of water.

2.14 Apply vacuum to the ENV column for approximately 30 seconds to remove aqueous trapped in the void spaces of the column.

2.15 Elute the analytes into a pre-calibrated 50-mL concentration tube using 10 mL of acetonitrile. (The concentration tube is calibrated by pipetting the desired final volume of sample diluent into the tube and then marking the meniscus with a marking pen. In these studies, a final sample volume of 5 mL was used for control samples and those fortified at the method LOQ of 0.5 ppb.) Add 0.2 mL of ethylene glycol to each concentration tube prior to rotary evaporation.

2.16 Remove the organic content from the sample via rotary evaporation with a water bath temperature of approximately 35 °C.

2.17 Add 1.0 mL of acetonitrile to the sample. Adjust the final volume to the calibration mark using purified water. The sample may be further diluted with sample diluent, if necessary. The sample should be stored refrigerated (<5°C) until the time of analysis. Samples should be stored frozen for long term storage (> 2 weeks).

2.18 Analyze the sample using LC/MS/MS.

E. Instrumentation

1.0 Description and Operating Conditions: HPLC

See Table 2 for a description of the reversed phase HPLC system and chromatographic conditions used for the analysis. The HPLC gradient has been slightly modified to lessen the overall analysis time.

2.0 Description and Operating Conditions: LC/MS/MS

CGA-362622, CGA-53052, CGA-382997, CGA-368732, CGA-368733, and NOA-436664 are monitored as positive ions. Triple quadrupole analysis (MS/MS) of the unique precurser/product ion pair is suggested to achieve the low LOQ of 0.5 ppb for all analytes. See Table 3 for a description of the mass spectrometer instrumentation and operating conditions.

3.0 Description and Operating Conditions: LC/MS/MS Turboionspray Interface

The optimized values for the turboionspray interface may vary with time and may need to be periodically re-optimized. The turboionspray split flow is adjusted so that a small wet spot is visible on the orifice plate at the initial mobile phase gradient composition. Typical turboionspray operating conditions are described in Table 3.

4.0 Calibration and Standardization: LC/MS/MS

Calibration and standardization procedure are the same as detailed in method AG-692. Typical precurser/product ion pairs that are monitored for each analyte are presented in Table 4.

F. Interferences

1.0 There are no known interferences originating from the sample cleanup procedure. However, interferences can originate from impure chemicals, solvents, contaminated glassware, and the HPLC water supply.

G. Confirmatory Techniques

1.0 No confirmatory analysis procedure is included in this method. This method employs highly specific LC/MS/MS for the detection mode, coupled with the characteristic retention time observed for the analyte on the appropriate HPLC column.

H. Time Required

1.0 The sample extraction and cleanup procedure can be completed for a set of ten samples in an eight-hour working day.

2.0 Each HPLC analysis requires approximately 21 minutes.

I. Modifications and Potential Problems

See modifications and potential problems detailed in method AG-692.

J. Preparation of Standard Solutions

The procedures for preparation of standard solutions are detailed in method AG-692.

K. Methods of Calculation

1.0 Determination of Residues in Samples

Same as detailed in method AG-692.

2.0 Determination of Residues in Fortified Samples

Same as detailed in method AG-692.

3.0 Calculations

Calculations may be performed by computer program or manually as follows (soil concentrations are based on their wet weight):

3.1 Calculate the analyte concentration (in ppb, wet weight) for field samples from equation (1):

$$(1)\ \text{ppb analyte (wet weight)} = \frac{\text{ng analyte found}}{\text{g sample injected}} \times \frac{1}{R}$$

where R is the recovery factor expressed in decimal form (i.e., 0.8 = 80%) and is calculated from equation (4), and the chemical purity of the analytical standard has been accounted for in the preparation of the standard solutions. The use of the recovery correction factor "1/R" is left to the discretion of the study director.

The mass of sample injected is calculated from equations (3) and (4), respectively.

The residue found may also be expressed on a dry weight basis by equation (2).

(2) $$\text{ppb analyte (dry weight)} = \frac{\text{ppb analyte (wet weight)}}{1-m}$$

Where "m" is the moisture percent of the soil sample expressed in decimal form (i.e., 15% soil moisture = 0.15).

The grams of sample injected for soil is calculated from equation (3).

(3) $$\text{g soil injected} = g \times \frac{V_i}{V_f}$$

where, g is the grams of soil (wet weight) used, V_i is the volume (mL) injected onto the HPLC column, and V_f is the final volume (mL) of the cleaned-up sample (from Step II.D.2.15).

The recovery factor, expressed as a percentage (R%), is calculated from fortification experiments and is presented in equation (4).

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

The amount (ppb) of analyte found is calculated from equation (5).

(5) $$\text{ppb analyte found} = \frac{\text{ng analyte found}}{\text{g sample injected}}$$

Residues of degradates found in test samples may also be expressed as parent equivalents by multiplying the amount found by the ratio of the molecular weight of CGA-362622 to that of the metabolite (equation (6)).

(6) $$\text{ppb CGA - 362622 equiv.} = \text{ppb metabolite} \times \frac{MW(p)}{MW(m)}$$

where MW(p) is the average molecular weight of CGA-362622 (437.3) and MW(m) is the average molecular weight of the metabolite, 155.2 for CGA-53052, 256.2 for CGA-382997, 373.3 for

CGA-368732, 330.3 for CGA-368733, and 316.2 for NOA-436664.

3.2 The accuracy of the method is determined by the average recovery of the analytes fortified into the test substrate. The precision is estimated by the relative standard deviation of the determined concentration.

3.3 The confidence limits at 95% were determined for each fortification level in each substrate using equation (7)

$$(7)\ \text{C.L.}(95\%) = X \pm \frac{ts}{\sqrt{n}}$$

where "X" is the mean recovery, "s" is the standard deviation, "n" is the number of samples, and "t" is the "t-test" value for (n-1) degrees of freedom. In this report, value of 3.182 was used for "t" with 3 degrees of freedom (for 4 samples), a value of 2.776 was used for 4 degrees of freedom (for 5 samples), a value of 2.179 was used for 12 degrees of freedom (for 13 samples), and a value of 2.145 was used for 14 degrees of freedom (for 15 samples), respectively, for the 95% confidence limit.

III. RESULTS AND DISCUSSION

The objective of Protocol 53-98[2] was to validate "Draft" Analytical Method AG-692 for the determination of CGA-362622 and its degradates CGA-53052, CGA-382997, CGA-368732, CGA-368733, and NOA-436664 in soil. The original, unmodified analytical method AG-692 was validated under Protocol 53-98 and Amendment 1. The results of these validation experiments were presented in analytical method AG-692 and residue test report RI-MV-011-98[3]. It became necessary to modify analytical method AG-692 due to observed low recoveries in soil samples from a field dissipation study being conducted in Louisiana. This final report amendment documents the modifications made to method AG-692 and provides supporting data that acceptable results are obtained with soil samples of varying depth from Louisiana that have been fortified, extracted, and analyzed using these method modifications.

The limit of determination (LOD) for all analytes was 0.0125 ng injected onto the column (25 µL x 0.0005 ng/µL). Adequate signal-to-noise ratios, which

permits accurate quantitation, were observed for all analytes. The absolute LOD obtainable will vary with the overall sensitivity of the LC/MS instrument, bandwidth of eluted peaks, and the overall optimization of the entire system. Good linearity for all analytes was routinely observed over the calibration range (0.0125 ng to 0.25 ng injected) with linear correlation coefficients typically exceeding 0.999. The linear dynamic range began to adversely deteriorate if the range was extended to 0.5 ng injected, as the detector response began to be saturated for some analytes.

The results from fortification experiments are presented in Tables 5-7 for Louisiana soils of depth zones of 0-6", 6-12", and 12-18". The tables contain raw data for both samples and calibration standards which permits the manual recalculation of recovery values. Attempts to duplicate the values in the tables will be subject to round-off errors. All calibration and residue calculations were performed using the Novartis Worksheet program, version 1.6.1. Peak areas were obtained using PE Sciex quantitation software, the peak areas were then manually entered into the Novartis Worksheet program, and the Worksheet program then performed the subsequent calculations. The recovery data from different sample sets and different fortification levels has been summarized in Tables 8-10 for Louisiana soils of depth zones of 0-6", 6-

Three sets (total of 15 samples) of soil samples were analyzed for the 0-6" soil zone fortified at 0.5 ppb due to marginal recoveries for CGA-382997 (68%, n=15). The average recovery improves to 70% if sample LA0-6-0.5-6 is omitted which had a non-typically low recovery of 46%. The average recoveries were quite good (84% and 99%) for CGA-382997 in the 0-6" soil layer at the higher fortification levels of 5 ppb and 100 ppb. Two of fifteen 0-6" samples fortified at 0.5 ppb (LA0-6-0.5-10 and LA0-6-0.5-15) exhibited non-typically enhanced recoveries (163% and 407%) for analyte CGA-368733. The cause of this enhancement is unknown and does not appear to be the result of laboratory contamination. This observation is non-typical and did not appear with any samples fortified at 5 ppb and 100 ppb or in samples from any other soil depth. The average recoveries (excepting CGA-382997 as previously noted) and percent relative standard deviations for all analytes at all fortification levels in 0-6" soil met the EPA criteria (70-120%, %RSD $\leq \pm 20\%$).

The average recoveries and percent relative standard deviations for all analytes at all fortification levels for the 6-12" soil layer met the EPA criteria, except NOA-436664 which had an average recovery of 68% at the 0.5 ppb LOQ level.

For the 12-18" soil layer, all analytes at all fortification levels met the EPA criteria except: (1) An average recovery of 67% was observed for

CGA-368732 at the 0.5 ppb LOQ level, (2) A percent relative standard deviation of 25.9% was observed for CGA-368733 at the LOQ level, and (3) A percent relative standard deviation of 32.2% was observed for CGA-382997 at the 5 ppb level (the %RSD meets the EPA criteria if a non-typical low recovery of 32% is omitted from the statistical calculations).

No interfering residues, or residues resulting from contamination, were observed in any of the method blank or control samples.

The Louisiana soils used in these additional validation experiments have been the most difficult soils we have encountered for obtaining acceptable recoveries of analytes fortified to the soils, requiring modifications to the original AG-692 extraction procedure. For several analytes at the LOQ of 0.5 ppb the average recoveries are marginal, being slightly less than the targeted minimum recovery of 70% (average recoveries of 68% for CGA-382997 in 0-6" soil, 68% for NOA-436664 in 6-12" soil, and 67% for CGA-368732 in 12-18" soil). Method AG-692 with the modifications described in this report has been tried on soils from different locations with very good recoveries observed for all analytes. The results from these non-GLP trials using soils from other locations will not be reported. This modified procedure is being successfully used to analyze samples from CGA-362622 field dissipation studies being conducted in Louisiana (Novartis study 42-99) and in California (Novartis study 40-99).

All raw data associated with this study and the original final report and protocol will be archived in the Agriculture Group Archives at Novartis Crop Protection, Inc., Greensboro, NC. All non-study specific data (i.e., instrument logbooks, etc.) will be stored in the previously mentioned archives when all entry pages are filled or when the logbook is replaced. Soil samples will be archived in the Sample Storage Facility, Greensboro, NC, until the registration studies have been accepted by the EPA. The Testing Facility (Novartis Crop Protection, Inc.) Quality Assurance Unit will verify the disposal of samples.

IV. CONCLUSION

Validation experiments have demonstrated that analytical method AG-692 with modifications is rugged, reliable, and accurate for the determination of CGA-362622, CGA-53052, CGA-382997, CGA-368732, CGA-368733, and NOA-436664 in soil. No interferences were observed of any of the analytes in method blank and control samples.

V. TABLES

TABLE 1. SOIL CHARACTERIZATION

	Soil from Louisiana		
Soil Depth	0-6"	6-12"	12-18"
pH	5.8	5.6	6.6
Cation Exchange Capacity (meq/100 g)	13.9	25.1	23.2
% Organic Matter	1.6	0.9	0.6
% Water Holding Capacity @ 1/3 Bar	23.1	32.9	29.6
% Water Holding Capacity @ 15 Bar	10.3	20.1	17.4
% Sand	21	15	23
% Silt	58	44	44
% Clay	21	41	33
Bulk Density (g/cc)	1.12	1.12	1.17
Soil Moisture Percent	14.3	28.1	19.2
USDA Textural Class	Silty Loam	Silty Clay	Clay Loam

All soil characterization data, except soil moisture percent, were determined under GLP guidelines by Agvise Laboratories, Northwood, ND. Soil moisture percentages were be obtained under Protocol 53-98.

TABLE 2. HPLC SYSTEM AND OPERATING CONDITIONS

Instrumentation:

Perkin-Elmer Series 200 Gradient Pump with Series 200 Vacuum Degasser
Perkin-Elmer Series 200 Autosampler with Peltier Sample Cooling Tray
Eppendorf Model CH-30 Column Heater

Operating Conditions:

Peltier Cooling Tray Temperature: 15°C
Column Heater: 30°C
Injection Volume: 25 µL
Mobile Phase Flow Rate: 0.6 mL/min
Column: MetaChem MetaSil AQ (MetaChem Technologies, Inc., #0530-150X030, 15-cm x 3.0 mm, dp = 5 µm, equipped with a MetaChem MetaGaurd 2.0 mm MetaSil AQ guard column (#0530-MG2)Upchurch (#A-318) pre-column filter (0.5 µm)
Mobile Phase A: 0.1% (v/v) acetic acid in acetonitrile
Mobile Phase B: 0.1% (v/v) acetic acid in purified water

Mobile Phase Gradient Program:

Time Duration (min.)	% A	% B	
-1 (equil.)	10	90	
0.0 (inject)	10	90	
10.0	70	30	linear ramp
0.1	100	0	linear ramp
3.0	100	0	
0.5	10	90	linear ramp
6.0	10	90	

Total Run Time: 20.6 min.

Approximate Analyte Retention Times:

CGA-53052	5.5 min
CGA-382997	6.2 min
CGA-368733	7.6 min
NOA-436664	9.1 min
CGA-368732	9.8 min
CGA-362622	10.8 min

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS

Instrumentation:

PE Sciex API-365 Triple Quadrupole Mass Spectrometer
TurbolonSpray Liquid Introduction Interface
Instrument Control and Data Collection: Apple MacIntosh Power PC Computer, Model 8500/180

Software:

Apple System 8.5

PE Sciex Software:

MassChrom v. 1.1.1
LC2Tune v. 1.4
Sample Control v. 1.4
MacDAD v. 1.4
MacQuan v. 1.6
Multiview v. 1.4
Bundler v. 1.4
File Translator v. 1.6.1
Downloader v. 1.2
Firmware (332) v. M3L1103
Firmware (340) v. M401100

Experiment Information:

Experiment Name: 362622 TIS-A
Scan Type: MRM
Scan Time: 0.510 sec/scan
Pause Time: 5.0 msec

Mass Range Info

	Q1 (amu)	Q3 (amu)	Dwell (msec)
Mass Range 1	156.1	100	250
Mass Range 2	257.0	176	250

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS
(Continued)

Experiment Name: 362622 TIS-B
Scan Type: MRM
Scan Time: 0.62 sec/scan
Pause Time: 5.0 msec

Mass Range Info

	Q1 (amu)	Q3 (amu)	Dwell (msec)	Ramp Parameters
Mass Range 1	331.1	248	150	none
Mass Range 2	317.1	234	150	none
Mass Range 3	374.1	331	150	R02 = -22.0 OR = 16.0
Mass Range 4	438.1	182	150	OR = 16.0

Method Information

Command	Description	Time (sec)	Duration (min)	Total Time (min:sec)
Pause		150	2.5	2:30
Scan Mode:	Profile	0.51	4.5	7:00
Threshold:	1.0 x 10 E1 cps			
Pause:	1.0 sec			
Expt:	362622 TIS-A			
State:	362622 State 1			
Scan Mode:	Profile	0.62	5.0	12:00
Threshold:	1.0 x 10 E1 cps			
Pause:	0.0 sec			
Expt:	362622 TIS-B			
State:	362622 State 2			

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS
(Continued)

Typical State File Values

362622 State 1

Positive Ions	
Gases	
NEB	6
CUR	10
CAD	5
Controls	
IS	5500
TEM	420
OR	15
RNG	85
Q0	-10
IQ1	-12
ST	-14
RO1	-12.0
IQ2	-16
RO2	-35.5
IQ3	-34.9
RO3	-45
DF	-200
CEM	1800

Q1 Resolution

Mass	Offset
30	0.038
100	0.051
1000	0.165
2000	0.280

Q3 Resolution

Mass	Offset
10	0.025
100	0.025
1000	0.120
2000	0.070

362622 State 2

Positive Ions	
Gases	
NEB	6
CUR	10
CAD	5
Controls	
IS	5500
TEM	420
OR	26
RNG	110
Q0	-10
IQ1	-12
ST	-15
RO1	-11.8
IQ2	-20
RO2	-41
IQ3	-60
RO3	-46
DF	-200
CEM	1800

Q1 Resolution

Mass	Offset
30	0.038
100	0.051
1000	0.165
2000	0.280

Q3 Resolution

Mass	Offset
10	0.025
100	0.020
1000	0.100
2000	0.070

* Note: State file values will vary slightly from instrument to instrument. The values often will be changed slightly during instrument optimization procedures.

TABLE 4. TYPICAL ANALYTE MONITORING IONS: LC/MS/MS

Analyte	Exact Molecular Weight	Q1 Molecular Ion	Q3 Product Ion
CGA-362622*	437.1	438.1	182.0
CGA-53052	155.1	156.1	100.0
CGA-382997	256.0	257.0	176.0
CGA-368732	373.1	374.1	331.0
		331.1**	248.0**
CGA-368733	330.1	331.1	248.0
NOA-436664	316.1	317.1	234.0

* Chemical standard exists as a monosodium salt, which has an exact molecular weight of 459.1.

** Extensive fragmentation of this molecule occurs during ionization, forming CGA-368733, which can then be monitored based on its unique Q1/Q3 pair. In these validation experiments the 331.1/248.0 ion transition was used for quantifying CGA-368732. This ion transition exhibits better signal-to-noise and better calibration linearity than the 374.1/331.0 transition.

TABLE 8. SUMMARY DATA FOR FORTIFIED LOUISIANA SOIL, 0-6" (cont.)

0.5 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	83	68	91	75	83	81
SD	9.7	10.1	11.7	7.9	6.5	7.7
RSD	11.8	14.8	12.9	10.6	7.9	9.5
n	15	15	13	15	15	15
C.L. (95%)	83 ± 5.4	68 ± 5.6	91 ± 7.1	75 ± 4.4	83 ± 3.6	81 ± 4.2

5 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	83	84	80	76	82	93
SD	4.2	5.6	5.4	2.1	3.6	4.9
RSD	5.1	6.6	6.7	2.8	4.4	5.3
n	5	5	5	5	5	5
C.L. (95%)	83 ± 5.2	84 ± 6.9	80 ± 6.7	76 ± 2.6	82 ± 4.4	93 ± 5.3

100 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	95	99	95	97	86	105
SD	3.2	3.0	4.0	3.6	5.2	3.3
RSD	3.4	3.0	4.3	3.8	6.1	3.2
n	5	5	5	5	5	5
C.L. (95%)	95 ± 4.0	99 ± 3.7	95 ± 5.0	97 ± 4.5	86 ± 6.5	105 ± 4.2

SD = Standard Deviation
RSD = Relative Standard Deviation
n = Number of Samples
C.L. (95%) = Confidence Limit at 95%

TABLE 9. SUMMARY DATA FOR FORTIFIED LOUISIANA SOIL, 6-12"

		% Recovery					
Sample Code	Fortification Level (ppb)	CGA-53052	CGA-382997	CGA-368733	NOA-436664	CGA-368732	CGA-362622
LA6-12-0.5-1	0.5	91	76	83	70	81	95
LA6-12-0.5-2	0.5	85	90	90	71	79	99
LA6-12-0.5-3	0.5	86	91	92	75	83	96
LA6-12-0.5-4	0.5	85	87	89	62	76	107
LA6-12-0.5-5	0.5	79	79	75	64	73	88
LA6-12-5-1	5	86	89	88	87	79	102
LA6-12-5-2	5	79	83	82	76	66	96
LA6-12-5-3	5	86	89	97	86	75	97
LA6-12-5-4	5	77	87	78	79	59	95
LA6-12-5-5	5	84	82	84	83	80	101
LA6-12-100-1	100	88	89	91	84	66	100
LA6-12-100-2	100	87	97	86	86	75	100
LA6-12-100-3	100	82	94	88	84	82	96
LA6-12-100-4	100	92	98	96	89	82	105
LA6-12-100-5	100	86	89	85	87	71	100

TABLE 9. SUMMARY DATA FOR FORTIFIED LOUISIANA SOIL, 6-12" (cont.)

0.5 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	85	85	86	68	78	97
SD	4.3	6.7	6.9	5.3	4.0	6.9
RSD	5.0	8.0	8.0	7.8	5.1	7.1
n	5	5	5	5	5	5
C.L. (95%)	85 ± 5.3	85 ± 8.4	86 ± 8.6	68 ± 6.6	78 ± 4.9	97 ± 8.6

5 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	82	86	86	82	72	98
SD	4.2	3.3	7.2	4.7	9.0	3.1
RSD	5.0	3.9	8.4	5.7	12.6	3.2
n	5	5	5	5	5	5
C.L. (95%)	82 ± 5.2	86 ± 4.1	86 ± 9.0	82 ± 5.8	72 ± 11.2	98 ± 3.9

100 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	87	93	89	86	75	100
SD	3.6	4.3	4.4	2.1	7.0	3.2
RSD	4.1	4.6	5.0	2.5	9.3	3.2
n	5	5	5	5	5	5
C.L. (95%)	87 ± 4.5	93 ± 5.3	89 ± 5.5	86 ± 2.6	75 ± 8.7	100 ± 4.0

SD = Standard Deviation
RSD = Relative Standard Deviation
n = Number of Samples
C.L. (95%) = Confidence Limit at 95%

TABLE 10. SUMMARY DATA FOR FORTIFIED LOUISIANA SOIL, 12-18"

12-18" Soil Layer

% Recovery

Sample Code	Fortification Level (ppb)	CGA-53052	CGA-382997	CGA-368733	NOA-436664	CGA-368732	CGA-362622
LA12-18-0.5-6	0.5	87	88	63	64	58	94
LA12-18-0.5-7	0.5	87	93	64	70	62	96
LA12-18-0.5-8	0.5	90	103	87	81	75	107
LA12-18-0.5-9	0.5	76	84	64	66	61	89
LA12-18-0.5-10	0.5	90	93	108	82	80	93
LA12-18-5-1	5	96	32	108	96	87	98
LA12-18-5-2	5	93	82	101	88	80	99
LA12-18-5-3	5	99	88	109	94	89	101
LA12-18-5-4	5	81	88	80	75	73	87
LA12-18-5-5	5	85	75	85	89	77	94
LA12-18-100-1	100	95	97	100	89	86	101
LA12-18-100-2	100	41*	40*	42*	38*	34*	41*
LA12-18-100-3	100	90	93	92	84	75	99
LA12-18-100-4	100	86	92	93	83	76	92
LA12-18-100-5	100	87	80	84	73	68	82

* Sample bumped violently several times during rotary evaporation. Sample will not be used for statistical calculations.

TABLE 10. SUMMARY DATA FOR FORTIFIED LOUISIANA SOIL, 12-18" (cont.)

0.5 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	86	92	77	73	67	96
SD	5.8	7.1	20.0	8.4	9.7	6.8
RSD	6.7	7.7	25.9	11.6	14.4	7.1
n	5	5	5	5	5	5
C.L. (95%)	86 ± 7.2	92 ± 8.8	77 ± 24.8	73 ± 10.4	67 ± 12.0	96 ± 8.4

5 ppb Fortification Level

	53052	382997	368733	436664	368732	362622
Mean	91	73*	97	88	81	96
SD	7.6	23.5*	13.4	8.2	6.7	5.5
RSD	8.3	32.2*	13.8	9.3	8.3	5.8
n	5	5	5	5	5	5
C.L. (95%)	91 ± 9.4	73 ± 29.2*	97 ± 16.6	88 ± 10.2	81 ± 8.3	96 ± 6.9

100 ppb Fortification Level

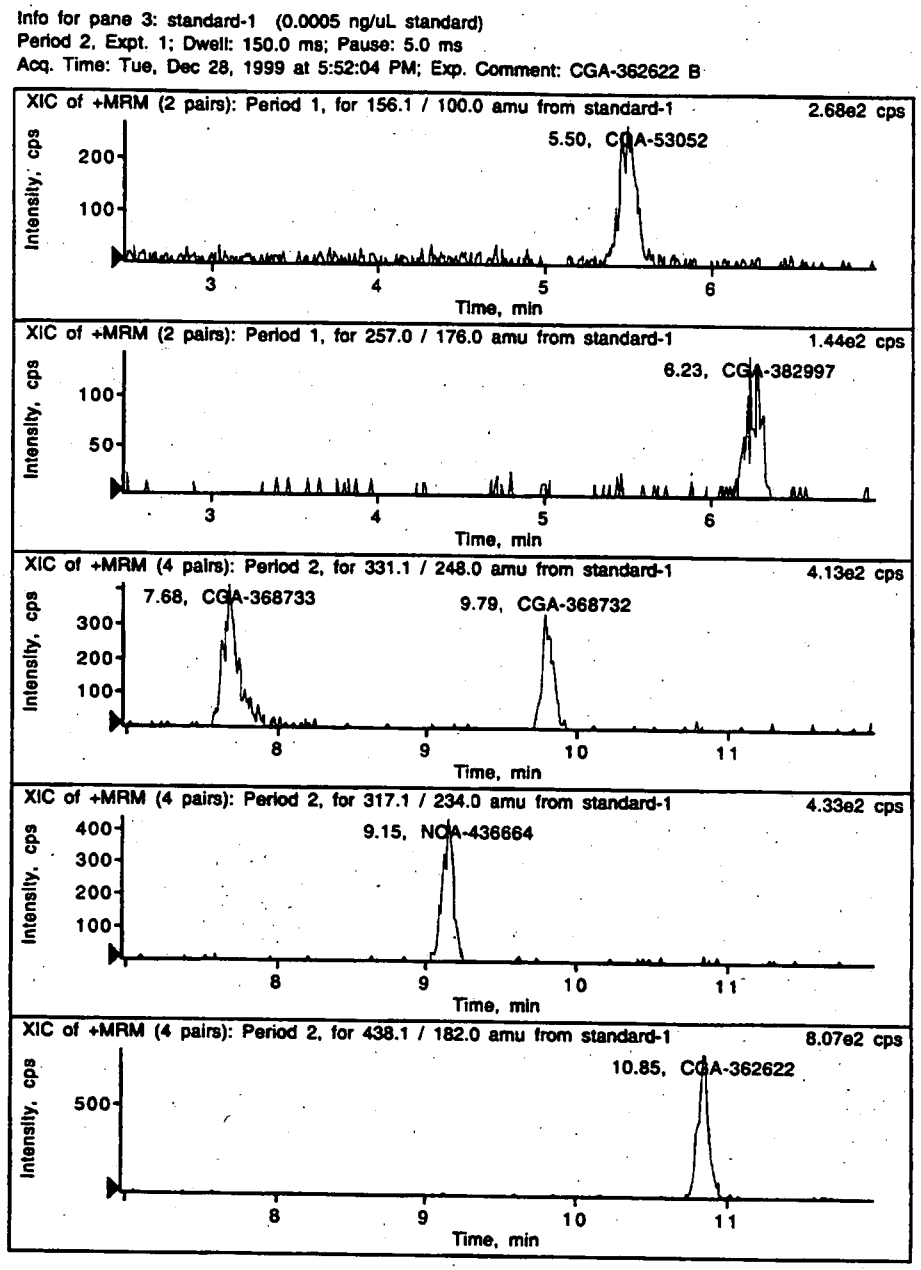
	53052	382997	368733	436664	368732	362622
Mean	90	91	92	82	76	94
SD	4.0	7.3	6.6	6.7	7.4	8.6
RSD	4.5	8.1	7.1	8.1	9.7	9.2
n	4	4	4	4	4	4
C.L. (95%)	90 ± 6.4	91 ± 11.7	92 ± 10.4	82 ± 10.7	76 ± 11.8	94 ± 13.7

* Statistical values skewed due to unexplained 32% recovery for one sample (LA12-18-5-1).

SD = Standard Deviation
RSD = Relative Standard Deviation
n = Number of Samples
C.L. (95%) = Confidence Limit at 95%

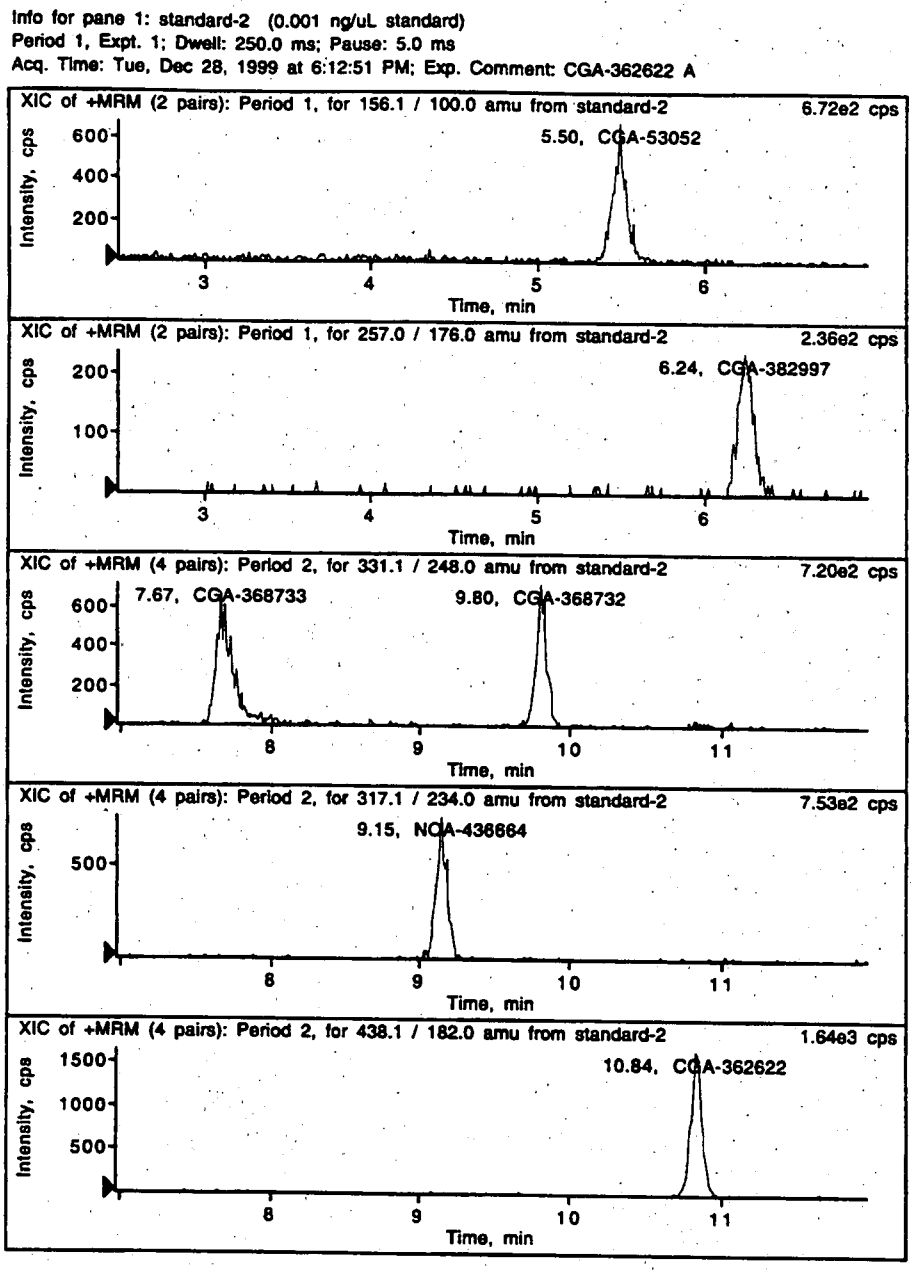
VI. FIGURES

FIGURE 1. TYPICAL CHROMATOGRAMS OF ANALYTICAL STANDARDS



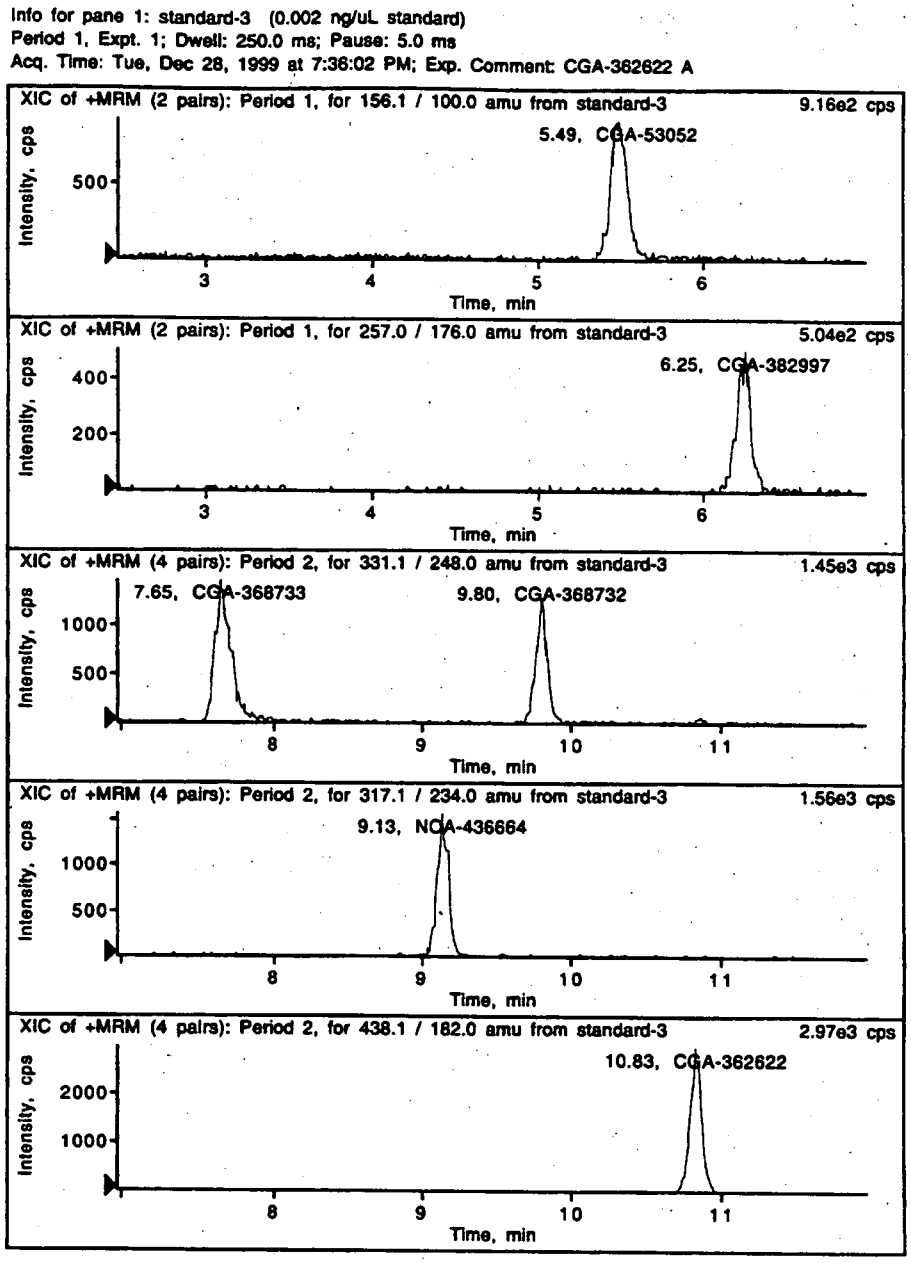
Low Standard: 0.0125 ng injected

FIGURE 1. TYPICAL CHROMATOGRAMS OF ANALYTICAL STANDARDS (cont.)



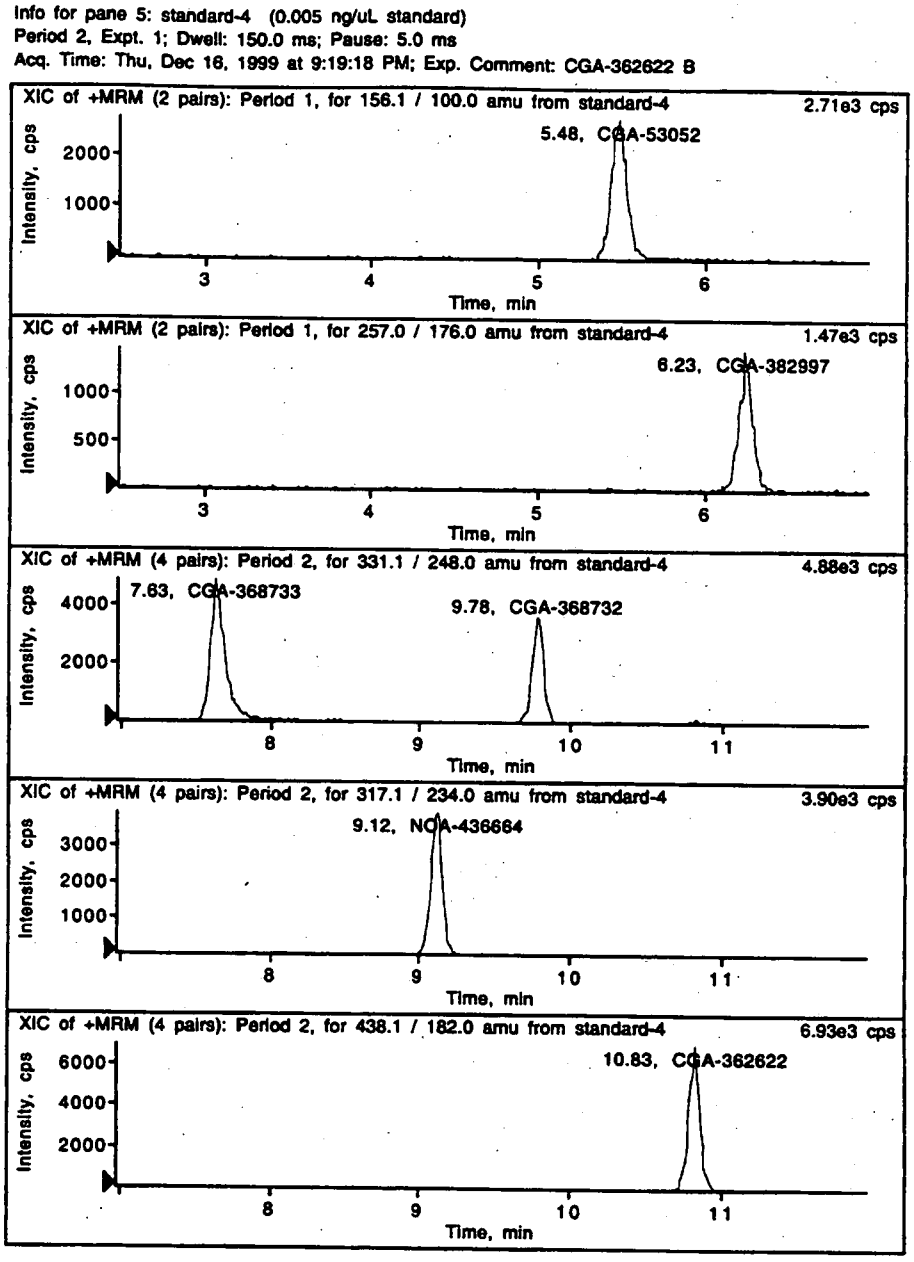
0.025 ng injected

FIGURE 1. TYPICAL CHROMATOGRAMS OF ANALYTICAL STANDARDS (cont.)

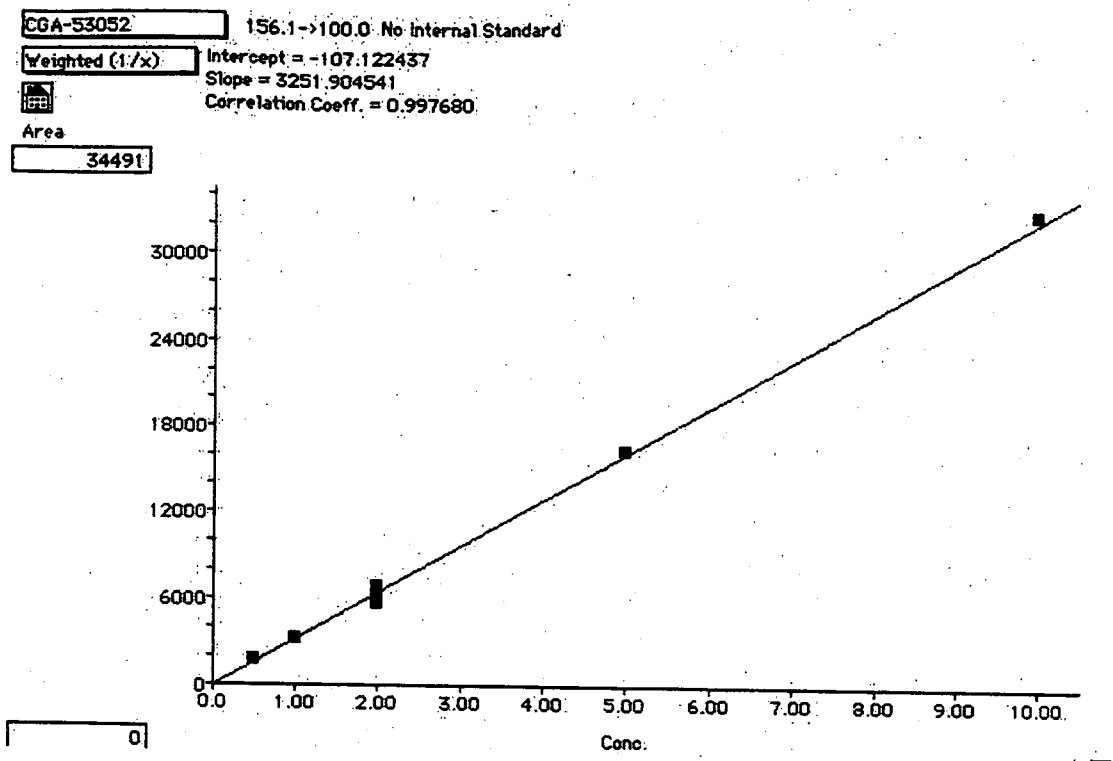


0.05 ng injected

FIGURE 1. TYPICAL CHROMATOGRAMS OF ANALYTICAL STANDARDS (cont.)



0.125 ng injected



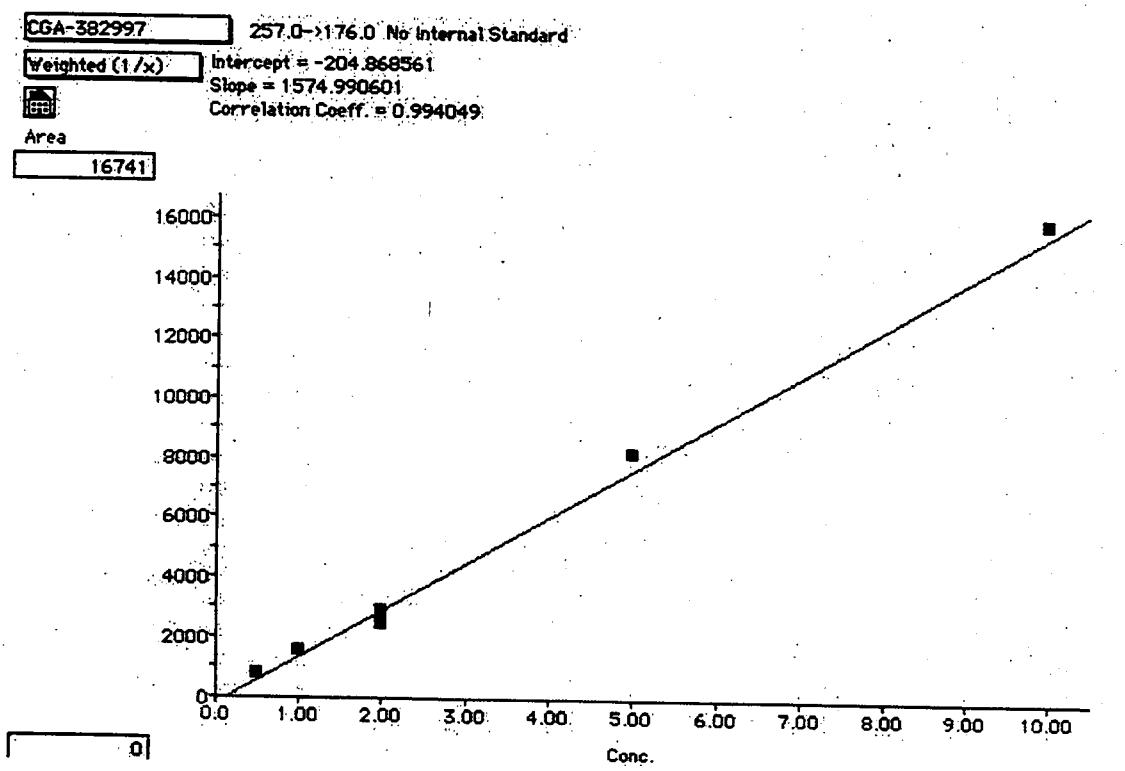


Figure 6. Calibration Curves for CGA-53052 and CGA-382997

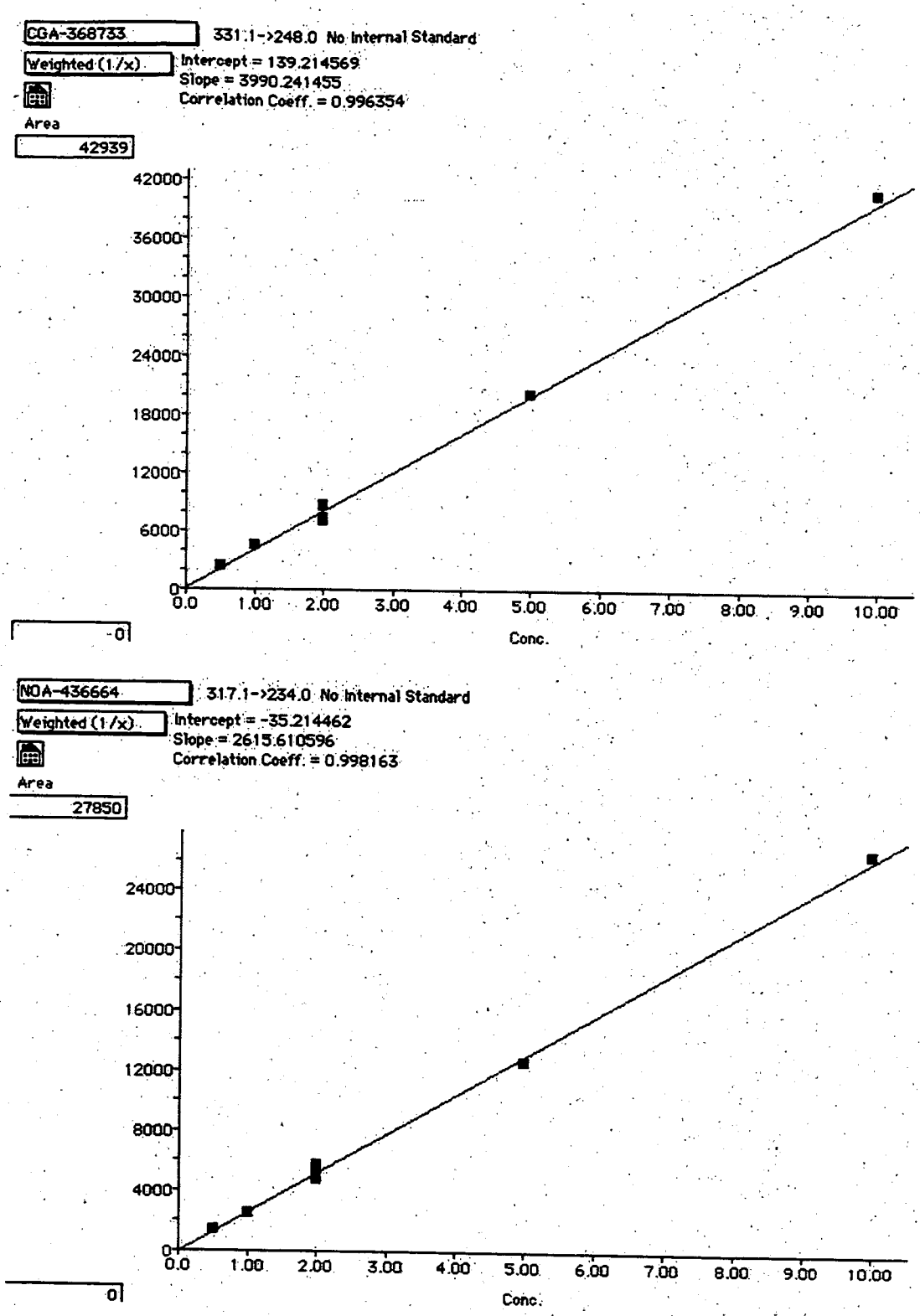
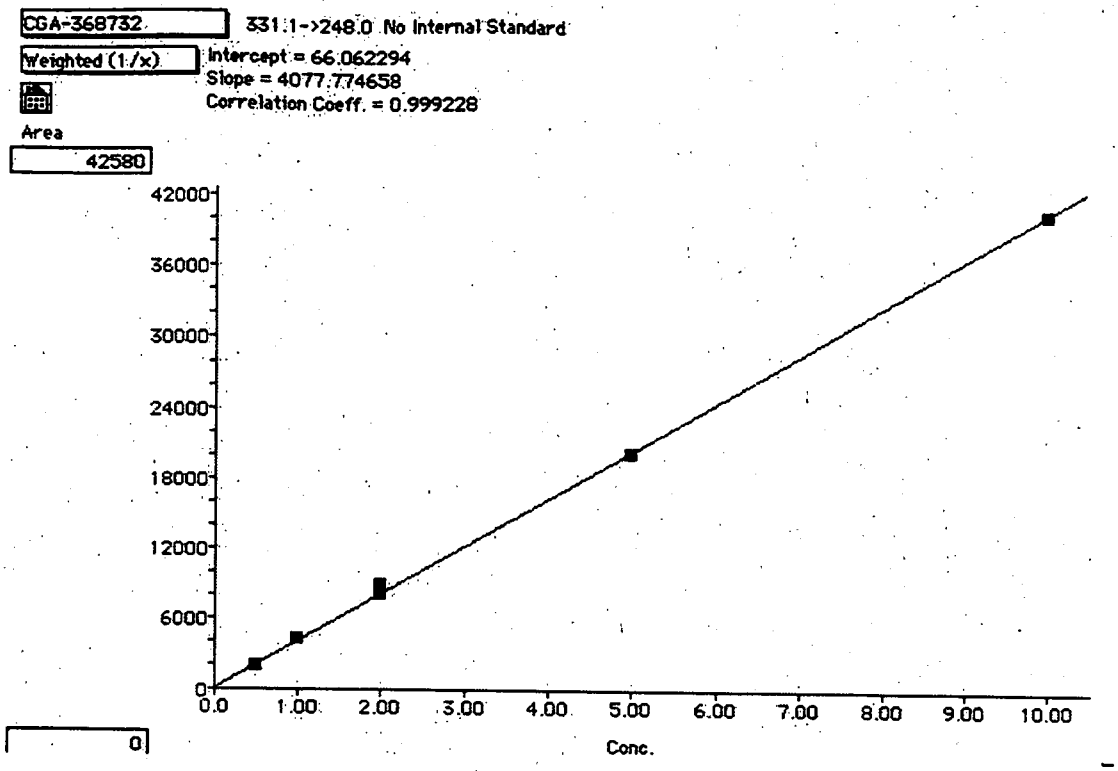


Figure 7. Calibration Curves for CGA-368733 and NOA-436664



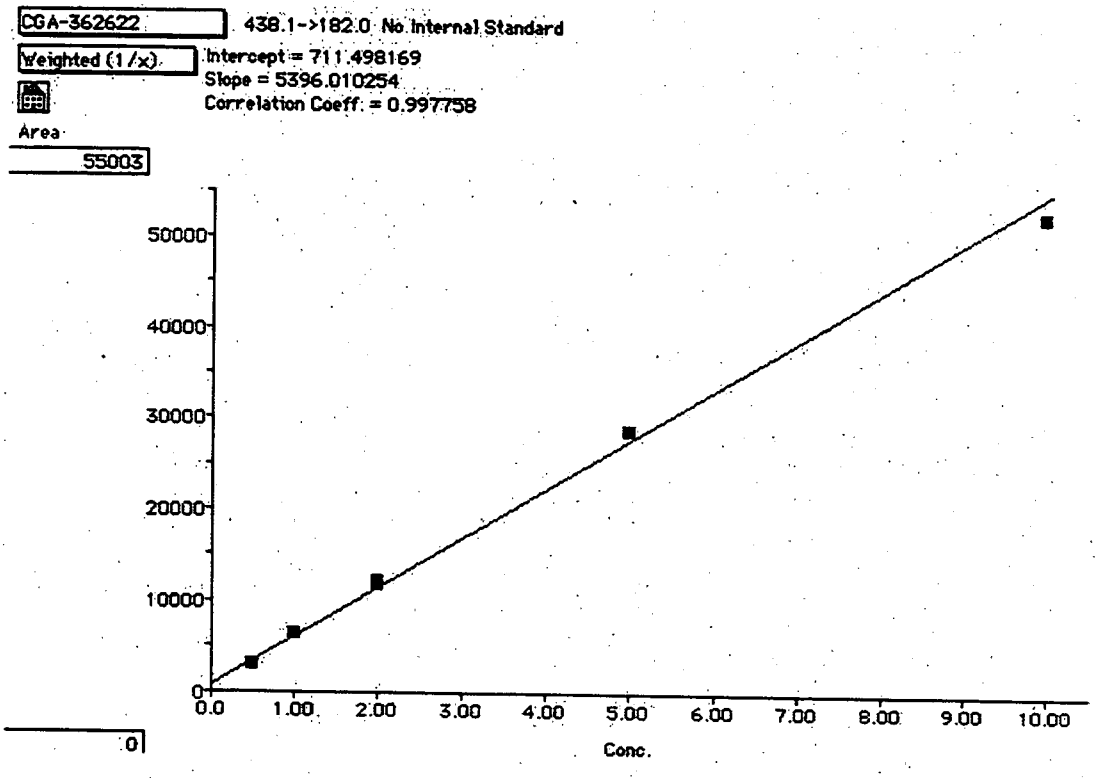


Figure 8. Calibration Curves for CGA-368732 and CGA-362622

CGA-53052 156.1->100.0 No Internal Standard

Weighted (1/x) Intercept = -107.122437

Slope = 3251.904541

Use Area Correlation Coeff. = 0.997680

Comments: Project 011978

Filename	Filetype	Sample Name	Conc.	Calc. Conc.	Area	Height
B061500001	Standard	0.01ug/mL	10.000	10.134	32848	3802
B061500002	Standard	0.005ug/mL	5.000	5.057	16337	1916
B061500003	Standard	0.002ug/mL	2.000	2.030	6494	801
B061500004	Standard	0.001ug/mL	1.000	0.991	3116	397
B061500005	Standard	0.0005ug/mL	0.500	0.550	1682	194
B061500006	Sample	Reagent Blank	n/a	n/a	n/a	n/a
B061500007	Sample	0-6" Control-1	n/a	n/a	n/a	n/a
B061500008	Sample	0-6" Control-2	n/a	n/a	n/a	n/a
B061500011	Standard	0.002ug/mL	2.000	2.131	6824	809
B061500009	QC	0-6" 0.5ppb QC-1	2.000	1.824	5826	740
B061500010	QC	0-6" 0.5ppb QC-2	2.000	1.805	5763	740
B061500012	QC	0-6" 0.5ppb QC-3	2.000	1.710	5454	720
B061500013	QC	0-6" 0.5ppb QC-4	2.000	1.810	5777	735
B061500014	QC	0-6" 0.5ppb QC-5	2.000	1.715	5469	703
B061500016	Standard	0.002ug/mL	2.000	1.753	5594	674
B061500015	QC	0-6" 5.0ppb QC-1	2.000	1.668	5317	698
B061500017	QC	0-6" 5.0ppb QC-2	2.000	1.697	5412	631
B061500018	QC	0-6" 5.0ppb QC-3	2.000	1.637	5215	656
B061500019	QC	0-6" 5.0ppb QC-4	2.000	1.649	5255	630
B061500020	QC	0-6" 5.0ppb QC-5	2.000	1.895	6055	782
B061500021	Standard	0.002ug/mL	2.000	1.853	5920	712

CGA-382997 257.0->176.0 No Internal Standard

Weighted (1/x) Intercept = -204.868561

Slope = 1574.990601

Use Area Correlation Coeff. = 0.994049

Comments: Project 011978

Filename	Filetype	Sample Name	Conc.	Calc. Conc.	Area	Height
B061500001	Standard	0.01ug/mL	10.000	10.253	15944	2111
B061500002	Standard	0.005ug/mL	5.000	5.302	8146	1113
B061500003	Standard	0.002ug/mL	2.000	1.979	2912	431
B061500004	Standard	0.001ug/mL	1.000	1.106	1536	228
B061500005	Standard	0.0005ug/mL	0.500	0.593	729	110
B061500006	Sample	Reagent Blank	n/a	n/a	n/a	n/a
B061500007	Sample	0-6" Control-1	n/a	n/a	n/a	n/a
B061500008	Sample	0-6" Control-2	n/a	n/a	n/a	n/a
B061500011	Standard	0.002ug/mL	2.000	1.843	2697	379
B061500009	QC	0-6" 0.5ppb QC-1	2.000	1.610	2331	311
B061500010	QC	0-6" 0.5ppb QC-2	2.000	1.639	2376	324
B061500012	QC	0-6" 0.5ppb QC-3	2.000	1.795	2623	359
B061500013	QC	0-6" 0.5ppb QC-4	2.000	1.499	2155	303
B061500014	QC	0-6" 0.5ppb QC-5	2.000	1.494	2149	284
B061500016	Standard	0.002ug/mL	2.000	1.738	2532	383
B061500015	QC	0-6" 5.0ppb QC-1	2.000	1.438	2059	282
B061500017	QC	0-6" 5.0ppb QC-2	2.000	1.354	1928	264
B061500018	QC	0-6" 5.0ppb QC-3	2.000	1.526	2199	326
B061500019	QC	0-6" 5.0ppb QC-4	2.000	1.608	2328	298
B061500020	QC	0-6" 5.0ppb QC-5	2.000	1.696	2467	328
B061500021	Standard	0.002ug/mL	2.000	1.687	2451	340

Figure 9. Raw Data for CGA-53052 and CGA-382997