Reference Substance Information:

Syngenta Code No.	CAS Number	CAS Name	Lot Number	Purity, %	Reassay Date
NOA-459602	not assigned	Sodium; 5-{5-methyl-4-[nitroimino][1,3,5]oxadiazinan-3-ylmethyl}-thiazole-2-sulfonate	DAH-XXIX-70	99.5	3/31/04
SYN-501406	not available	Sodium; 5-(N'-Methyl-N"-nitro-guanidinomethyl)-thiazole-2-sulfonate	DAH-XXX-2	74.0	6/30/04
CGA-293343	153719-23-4	4*H*-1,3,5-Oxadiazin-4-imine, 3-[(2-chloro-5-thiazolyl) methyl]tetrahydro-5-methyl-*N*-nitro-	DAH-XXIX-7	99.6	5/31/05
CGA-322704	131748-59-9	Guanidine, *N*-[(2-chloro-5-thiazolyl)methyl]-*N*'-methyl-*N*"-nitro-	DAH-XXVII-52	96.6	5/31/05
CGA-355190	not available	4H-1,3,5-Oxadiazin-4-one, 3-[(2-chloro-5-thiazolyl) methyl] tetrahydro-5-methyl-	DAH-XXVI-63	97.6	9/30/04
NOA-404617	not available	Urea, *N*-{(2-chloro-5-thiazolyl)methyl]-*N*'-nitro-	DAH-XXVIII-31	99.9	9/30/04
CGA-353042	not available	2*H*-1,3,5-Oxadiazin-4-amine, 3,6-dihydro-3-methyl-	JAK-XX-52	97.1	6/30/04
NOA-407475	not available	4*H*-1,3,5-Oxadiazin-4-imine, 3-[(2-chloro-5-thiazolyl) methyl]tetrahydro-5-methyl-	DAH-XXVIII-46	95.5	9/30/04

ABBREVIATIONS AND SYMBOLS

Abbreviation	Definition
A	acre
a.i.	active ingredient
amt	amount
amu	atomic mass unit
C	Celsius or Centigrade
CAS	Chemical Abstract Services
CFR	Code of Federal Regulations
cm	centimeter
DA[#]A	days after application, [#] = 1, 2, 3 etc., if there are multiple applications
EPA	Environmental Protection Agency (U.S.)
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (U.S.)
ft	foot (feet)
g	gram
gal	gallon
GC	gas chromatography
GLPs	Good Laboratory Practices
ha	hectare
HPLC	high performance liquid chromatography
i.d.	inside diameter
ID	identification
in.	inch
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
L	liter
lb	pound
LC	liquid chromatography
LC/MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
m	meter
m/z	mass to charge ratio
µg	microgram

ABBREVIATIONS AND SYMBOLS (continued)

Abbreviation	Definition
µL	microliter
µm	micrometer
MDL	method detection limit
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mmol	millimole
mol	mole
MS	mass spectrometry
MS/MS	tandem mass spectrometry/mass spectrometry
mV	millivolt
MW	molecular weight
N/A	not applicable
ND or nd	nondetect (below limit of detection)
ng	nanogram
No.	number
oz	ounce
PMRA	Pest Management Regulatory Agency, Canada
ppb	parts per billion or micrograms per kilogram
ppm	parts per million or microgram per gram or milligrams per kilogram
pg	picogram
psi	pounds per square inch
QAU	quality assurance unit
R^2 (or r^2)	square of correlation coefficient
RSD	relative standard deviation
Rt	retention time
s	second
SD	standard deviation
USDA	United States Department of Agriculture
UV	ultraviolet
vol	volume
wt	weight

1.0 INTRODUCTION/SUMMARY

1.1 Scope

This method is used for the determination of CGA-293343 (Chemical Abstracts Registry (CAS) Number: 153719-23-4, 4*H*-1,3,5-Oxadiazin-4-imine 3-[(2-chloro-5thiazolyl) methyl]tetrahydro-5-methyl-*N*-nitro-, and its degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in water. The compounds are separated by high performance liquid chromatography (HPLC) and detected by mass spectrometry (LC/MS/MS). An Ion-Spray atmospheric pressure ionization (API) interface is used to introduce the HPLC effluent into the mass spectrometer. The analytes are detected in the triple quadrupole mode (MS/MS) by passing the positive/negative molecular ion through Q1, inducing fragmentation in Q2, and then monitoring a characteristic product ion in Q3. The chemical structures, chemical names, and Chemical Abstracts Registry numbers of the analytes are presented in FIGURE 1.

The limit of detection by LC/MS/MS (smallest standard amount injected during the chromatographic run) is 1.25 pg for all analytes. The limit of determination (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for LC/MS/MS analyses is 0.05 ppb for all analytes in water.

This is an alternate method to methods 442-00 and RAM 396/01 previously validated for the determination of CGA-293343 and its degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in water.

1.2 Principle

An aliquot of the sample is acidified to 0.1% acetic and transferred into an HPLC autosampler vial for analysis by LC/MS/MS.

2.0 MATERIALS AND METHODS

2.1 Apparatus

1) Balance, analytical (Sartorius R160P) or equivalent.

2) Pasteur pipette (Fisher cat. #13-678-7C) or equivalent.

3) Cylinder, graduated, 100-mL, 50-mL, 25-mL, and 10-mL (Fisher cat. #08-550E, 08-550D, 08-550C and 08-551B) or equivalent.

4) Pipettes, glass, class A certified, assorted volumes. These pipettes are used when an exact addition of liquid is required (i.e., dilution of standards).

5) Pipetters, Oxford BenchMate adjustable, 40-200 µL volume range (Fisher cat. #21-231), 200-1000 µL volume range (Fisher cat. #21-229) or equivalent. (Note: These adjustable pipetters may only be used for addition of liquid where an exact volume added is not critical, i.e., addition of acid or base.).

6) Vials, clear or amber, 1.5-mL (Sun Brokers, Inc. cat. #200-002) or equivalent, with Teflon-lined, crimp-top seals (Sun Brokers, Inc. cat. #200-152) or equivalent.

2.2 Reagents and Analytical Standards

All reagents and polypropylene glycols are stored at room temperature. Solid analytical standards are stored in a freezer (temperature <-10°C).

1) Acetic acid, concentrated, HPLC grade (Fisher cat. #A35-500) or equivalent.

2) Acetic acid, 0.1% solution: Mix 1 mL of acetic acid with 999 mL of purified water.

3) Acetonitrile, HPLC grade (Fisher cat. #A998-4) or equivalent.

4) Ammonium acetate, HPLC grade (Fisher cat.#A639-500) or equivalent.

5) Methanol, HPLC grade (Fisher cat. #A452-4) or equivalent.

6) Polypropylene glycol, M.W. 425 (Aldrich cat. #20,230-4).

7) Polypropylene glycol, M.W. 1000 (Aldrich cat. #20,232-0).

8) Polypropylene glycol, M.W. 2000 (Aldrich cat. #20,233-9).

9) PPG tuning solution (for mass calibration of the LC/MS/MS system). Dissolve 0.0014 g PPG 425, 0.0100 g PPG 1000, 0.0400 g PPG 2000, and 0.0126 g of ammonium formate in 50 mL of methanol, 50 mL water, and 0.1 mL of acetonitrile. Mix well. Store refrigerated in an amber bottle. Dilute 1/50 with 1:1 MeOH:water before use.

10) Sample diluent: 5% methanol/water + 0.1% acetic acid. Mix 50 mL of methanol with 950 mL water and 1 mL of acetic acid.

11) Test analytes tuning solution, 0.05 ng/µL. Mix 1 mL of a 1 ng/µL mixed solution of analytes in acetonitrile with 19 mL of 50% acetonitrile/water with 0.1% acetic acid. Store at refrigerated temperature.

12) Water, HPLC grade, purified in-house with a HYDRO™ purification system or equivalent.

13) Ammonium acetate, 25 mM: Mix 1.927 g of ammonium acetate with 1 L of purified water.

14) Mobile phase A: 0.1% acetic acid in water. Mix 1 mL of acetic acid with 999 mL of purified water.

15) Mobile phase B: 0.1% acetic acid in methanol. Mix 1 mL of acetic acid with 999 mL of methanol.

16) Mobile phase C: 50% acetonitrile/25 mM ammonium acetate. Mix 500 mL of acetonitrile with 500 mL of 25 mM ammonium acetate.

17) CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300. Standard solutions should be prepared from stock solutions (organic) every three months and stored refrigerated.

2.3 Safety and Health

Whereas most of the chemicals used and analyzed in this method have not been completely characterized, general laboratory safety is advised (e.g., safety glasses, gloves, etc. should be used).

3.0 ANALYTICAL PROCEDURE

Note: All glassware should be thoroughly cleaned and followed with a rinse of acetonitrile or methanol prior to use. The analysis system is very sensitive and may detect contamination from previous samples if all glassware is not properly cleaned prior to each use.

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

1) Aliquot a volume of sample and acidify to 0.1% acetic with acetic acid.

2) Sample fortification, if required for this particular sample, is to be done at this time.

3) Transfer the sample into an autosampler vial and analyze by LC/MS/MS system I with a reversed-phase HPLC as detailed in Table I for the presence of CGA-293343, CGA-322704, CGA-355190, and NOA-404617, NOA-459602 and SYN-501406.

4) Use LC/MS/MS system II with a cation exchange HPLC as detailed in Table II to determine CGA-353042 and NOA-407475.

5) A six-port electric-actuated column switching valve (Valco or equivalent) may be used to connect both analytical columns between the autosampler and the MS interface to switching the column automatically (FIGURE 5).

4.0 INSTRUMENTATION

4.1 Description and Operating Conditions: HPLC

See Table I and II for a description of the HPLC system and chromatographic conditions. These tables also describe typical MS state file values and for conditions used with the Turbo Ion-Spray interface of API-4000 in the Analytical Method 3103-03. The optimized values for the mobile phase may be adjusted from time to time. Different HPLC columns that give comparable separation may be used. The proposed LC condition used here is optimized for sensitivity and to minimize matrix suppression effect. A shorter column may be used if matrix suppression and sensitivity are not the issue to save solvent and cut the analysis time.

4.2 Description and Operating Conditions: LC/MS/MS

CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617 and NOA-407475 are monitored as positive ions. SYN-501406 and NOA-459602 are monitored as negative ions. Triple stage quadrupole analysis (MS/MS) of the unique precursor/product ion pair is suggested, although single stage quadrupole analysis (MS) utilizing the molecular ion may be performed provided that no interferences are present in the sample matrix. The optimized values for the analytes state files may vary with time and need periodic re-optimization by infusion of the analytes into the mass spectrometer. Using a different instrument with a different MS interface may result in a different intensity of product ions and the acquisition method file must be adjusted accordingly.

4.3 Calibration and Standardization: LC/MS/MS

- Calibrate and tune the mass spectrometer prior to analyzing samples. Check the calibration and tune by infusing a standard solution of polypropylene glycol (PPG) into the mass spectrometer using the Turbo Ion-Spray interface while monitoring positive ions. A typical mass calibration tune with PPG is shown in FIGURE 3. Weekly calibrations and tunes with the PPG solution are considered sufficient provided that instrument mass calibration stability is demonstrated for that time interval.

- Determine the specific ion to monitor for each analyte by infusion of an analyte test solution (approx. 0.05 ng/µL in 50% acetonitrile/water, 0.1% acetic acid) while scanning the Q1 quadrupole mass analyzer to find the optimum ion. Determine the specific product ion fragment to monitor for each analyte in the MS/MS mode by passing the characteristic precursor ion through Q1, fragmenting the ion in Q2, and scanning the resulting ion fragments in Q3. The selected product ion chosen to monitor will depend on the intensity of the ion fragment along with the possibility that an interference also has the same fragment ion. Typical Turbo Ion-Spray mass fragmentation spectra are presented in FIGURE 4.

- Determine the retention time of the analytes by injecting a standard solution into the HPLC. During a series of analyses, the analyte retention time should vary no more than 2% from its mean value, on a daily basis.

- Calibrate the instrument by constructing a calibration curve from detector response (chromatographic peak height or area) and the amount of analyte injected, encompassing a range from 1.25 to 50 pg (50 µL injection) for Develosil column and from 0.5 to 20 pg (20 uL injection) for Zorbax SCX column. The response curve can be constructed manually or, preferably, by generation of a linear regression equation by use of a computer or appropriate calculator. Typical calibration data and chromatograms of calibration standards are presented in TABLEs 3-8 and FIGUREs 6-7.

5.0 INTERFERENCES

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

6.0 CONFIRMATORY TECHNIQUES

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 anlayte on the appropriate HPLC column.

7.0 TIME REQUIRED

For a set of 12 samples, sample aliquot and data compilation can be completed in an eight-hour working day.

Each HPLC analysis requires approximately 10-18 minutes.

8.0 MODIFICATIONS AND POTENTIAL PROBLEMS

1) Contaminants from chemicals, solvents, glassware, and the HPLC water supply can interfere with the analysis. It is recommended that a reagent blank be run with an analysis set to verify that no interferences are originating from the chemicals and reagents used in this procedure. MS techniques are very sensitive. All glassware should be solvent rinsed before use to prevent inadvertent contamination of control or low level samples.

2) Analytical Method 3103-03 was validated only for the water type listed in the final method. Other water samples from different locations may exhibit interference problems which were not observed with these samples.

3) No analyte stability or solubility problems have been observed when solutions have been prepared and stored as detailed in Section 9.

4) Long-term optimization of the LC/MS/MS signal by infusion of a test mixture of analytes into the system will result in lingering high backgrounds for the molecular ions. While the background signals will decrease with time or cleaning of the orifice plate, it may be severe enough to affect the ability to achieve desired signal to noise ratios for lowest standards. For this reason it is highly recommended that optimizing/calibrating with analytical standards be done with dilute solutions and the optimizing/calibrating time be minimized. It is also recommended after calibrating/optimizing with test analytes, to turn the power off to the electronics, remove the Turbo Ion-Spray interface, and thoroughly wipe clean the orifice plate using a lint-free tissue wetted with methanol. Repeat several times.

5) Equilibrate the analytical column before sample analysis by injecting at least 5 injections of standard solution at the LOQ to establish consistent retention time and sensitivity.

6) This method has been tested only on a PE Sciex API-4000 LC/MS/MS system using the Turbo Ion-Spray interface. Different brand or model of LC/MS/MS system with Turbo Ion-Spray interface may be used if the sensitivity of all analytes are acceptable.

7) If the samples are not to be analyzed in the same day, it should be stored in a refrigerator to prevent analyte degradation, particularly for CGA-353042. The sample should be analyzed within 3-5 days of preparation.

8) The cation exchange HPLC column may be cleaned of bound components by passing 25 mM ammonium acetate through the column for approximately 30 minutes. The column should then be equilibrated for approximately 30 minutes with the mobile phase used for analysis prior to starting a new sample run sequence. It is recommended to perform this cleaning procedure on a frequent basis. After finishing the analysis, the column should be flushed with 20% MeOH/water for approximately 15 min at a flow rate of 1 mL/min to wash away salt residues. The column is then flushed with MeOH at a flow rate of 1 mL/min for 15 min and stored in MeOH.

9) Reversed-phase columns from other manufacturers may be substituted for the column used in this study provided that the analyst demonstrates acceptable peak shape and sensitivity.

10) To prevent the effect of matrix to the analytes response (signal suppression), inject the least amount of sample that will give a satisfactory response (signal/noise at least 10). Sample with high concentration of humic acid, particularly surface water, seems to have more signal suppression effect than the cleaner water like ground water.

11) The sample is directly analyzed without any filtration. If the sample is turbid, it should be centrifuged at the appropriate speed to obtain a clear supernatant. It's not recommended to filter the sample as the analytes may be absorbed on the filter. Pre column filter (just before the analytical column) may be replaced as needed i.e. after a few sets if the water sample has a lot of fine particulates remaining in the supernatant.

9.0 PREPARATION OF STANDARD SOLUTIONS

All individual standard solutions are stored in amber bottles in a refrigerator (< 5°C) when not in use. Mixed standards solutions are also stored in a refrigerator. No analyte stability problem has been observed in the standard solutions used in this study. The mixed standards are used for fortifications and calibration standards.

Prepare individual 100 ng/µL stock solutions for CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-407475, NOA-404617, SYN-501406 and NOA-459602. Weigh approximately 10.0 mg of analyte. Determine the appropriate volume of solvent to add using the equation presented below. The concentration of the analytical standard is corrected for its chemical purity. Acetonitrile is used as the solvent for CGA-293343, CGA-322704, and CGA-355190. CGA-353042, NOA-407475, SYN-501406, NOA-459602 and NOA-404617 are dissolved in 30% water/acetonitrile because they do not dissolve well in 100% acetonitrile at high concentration.

$$V\,(mL) = \frac{W(mg) \times P}{C\,(ng/\mu L)} \times 10^3$$

Where V is the volume of solvent needed; W is the weight, in mg, of the solid analytical standard; P is the purity, in decimal form, of the analytical standard; C is the desired concentration of the final solution, in ng/µL; and 10^3 is a conversion factor.

For example:

The volume required to dilute 9.9 mg of an analyte, of 98.0% purity, to a final concentration of 100 ng/µL is:

$$V\,(mL) = \frac{9.9\,mg \times 0.98}{100ng/uL} \times 10^3 = 97.02\,mL$$

Fortification and calibration standards are prepared from the analyte stock solutions. Prepare a 10 ng/µL mixed solution by pipetting 10.0 mL of each analyte 100 ng/µL stock solution into a 100-mL volumetric flask and then diluting to the calibration mark with 5% methanol/water + 0.1% acetic acid. Subsequent dilutions of this solution with 5% methanol/water + 0.1% acetic acid will depend upon the desired fortification level(s). Fortification standards should be prepared such that no more than 1.0 mL of the fortification

solution is added to a sample. (Example: For a 100 mL water sample, the addition of 0.5 mL of a 0.01 ng/µL fortification solution will result in a fortification level of 0.05 ppb.)

Prepare a 0.1 ng/µL mixed standard for generating external calibration curves for CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 on the LC/MS/MS system. Pipette 1.0 mL from the 10 ng/µL mixed standard solution into a 100-mL volumetric flask and dilute to the calibration mark using 5% methanol/water + 0.1% acetic. Subsequent dilutions using 5% methanol/water + 0.1% acetic are made to prepare a series of calibration standards.

10.0 METHODS OF CALCULATION

10.1 Determination of Residues in Samples

Inject the sample solution from Step 3.2 into the LC/MS/MS system. The sample solution may be diluted if the analyte response exceeds the range of the calibration curve. The amount of analyte injected (pg) is determined by entering the value of the chromatographic peak height or area, in the calibration response curve and calculating (by computer, calculator, or manual means) the corresponding value of nanograms injected. Typical chromatograms for fortified water are presented in FIGURE 8-15.

10.2 Determination of Residues in Fortified Samples

Validate the method for each set of samples analyzed by including a control sample and one or more control samples fortified prior to the extraction procedure with 0.05 ppb or more of each analyte in water.

Add an appropriate volume of a fortification solution to the sample prior to injection. The total volume of the added fortification solution should not exceed 1.0 mL.

Proceed with the sample injection.

10.3 Calculations

Calculations may be performed by computer program or manually as follows:

Calculate the analyte concentration (in ppb) for field samples from equation (1):

$$(1)\ \text{ppb analyte} = \frac{\text{pg analyte found}}{\text{mg sample injected}} \text{ X } \frac{1}{\text{R}}$$

where R is the recovery factor expressed in decimal form (i.e., 0.8 = 80%) and is calculated from equation (2), 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. One gram of water is equal to 1 mL of water.

$$(2)\ R\% = \frac{\text{ppb analyte found - ppb analyte (control)}}{\text{ppb analyte added}} \text{X}\,100\%$$

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.

If background interference is found in the matrix blank (water control), it will be reported in the data table. In addition, an indication must be made as to whether or not these amounts were taken into account in the recovery calculations. The decision of whether to subtract any amounts found in the matrix blank from the recovery sample(s) is left to the discretion of the study director.

12.0 CONCLUSION

This analytical method, Method 3103-03, was successfully shown to be an accurate and reliable procedure for the determination of CGA-293343 and its degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, NOA-459602, and SYN-501406 in water. This method was validated at the LOQ of 0.05-ppb and ten times the LOQ using control water from surface (City Lake and Buffalo Creek) and ground water. Average recoveries were within the EPA guidelines for recovery (70-120%) and precision (RSD $\leq$ 20%) at each fortification level and for all eight analytes.

13.0 TABLES AND FIGURES

TABLE 1. LC/MS/MS SYSTEM I AND OPERATING CONDITIONS

This system is used for the determination of NOA-459602, SYN-501406, CGA-293343, CGA-355190, CGA-322704, and NOA-404617.

HPLC Instrumentation:
Perkin-Elmer Series 200 Quaternary Gradient Pump
Perkin-Elmer Series 200 Autosampler
Perkin-Elmer Series 200 Column Heater

Operating Conditions
Column Heater: 40 °C
Injector Volume: 50 µL (50-100 uL depending upon instrument sensitivity)
Mobile Phase Flow Rate: see below
Column: C-18 guard column (cat# AJO-4287, Phenomenex) equipped with a guard column holder (cat# KJO-4282, Phenomenex)
Develosil RP Aqueous, 3µm 150x3 mm (cat# CH0-6001 Phenomenex),
An Upchurch (A-318) pre-column filter (0.5 µm) is also installed between the autosampler and columns to prevent fine particles from the sample blocking the columns.
Mobile phase A = 0.1% acetic acid in water
B = 0.1% acetic acid in methanol
Mobile Phase Gradient Program:
LC Flow into the Mass Spectrometer with **no** split

Time (min.)	% A	% B	Curve	Flow rate (mL/min)
0	95	5		0.6
1	95	5		0.6
3	0	100	1	0.6
5	0	100		0.6
8	95	5	0	1.0
0.5	95	5	0	0.6

Total Run Time: 18 min
Analyte Retention Times:

NOA-459602	3.87 min
SYN-501406	6.04 min
CGA-293343	6.83 min
CGA-322704	7.14 min
CGA-355190	7.27 min
NOA-404617	7.57 min

TABLE 1. LC/MS/MS SYSTEM I AND OPERATING CONDITIONS (CONTINUED)

MS Instrumentation:

PE Sciex API-4000 Triple Quadrupole Mass Spectrometer
Instrument Control and Data Collection: Dell Computer, Model Precision 340

Software: Analyst 1.3

All software programs are written and provided by PE Sciex.
Different versions of the system and applications software may be used provided they are able to collect and process the data properly.

Period 1. Last for 2.5 min

This period is a delay period where the ion-spray voltage is set to 0 volt so that none of compounds in the HPLC eluent, (particularly the early eluting polar organic compounds and salts) is charged; therefore, they do not enter the mass spectrometer and keep the instrument clean.

Operating Conditions:

Interface	:	Turbo Ion-Spray
Polarity	:	Negative
Nebuliser Gas (GS1)	:	50
Turbo Gas (GS2)	:	50
Curtain gas (CUR)	:	15 (arbitrary units)
Temperature (TEM)	:	600
Ion-Spray voltage	:	0
Collision gas (CAD)	:	Nitrogen 3.0 (arbitrary units)
Scan type	:	MRM

TABLE 1. LC/MS/MS SYSTEM I AND OPERATING CONDITIONS (CONTINUED)

Period 2. Last for 2.5 min

This period is used to monitor NOA-459602 in a negative mode.

Operating Conditions:

Interface	:	Turbo Ion-Spray
Polarity	:	Negative
Nebuliser Gas (GS1)	:	50
Turbo Gas (GS2)	:	50
Curtain gas (CUR)	:	25 (arbitrary units)
Temperature (TEM)	:	600
Ion-Spray voltage	:	-3500
Collision gas (CAD)	:	Nitrogen 4.0 (arbitrary units)
Scan type	:	MRM

Analyte	NOA-459602
Q1 mass	335.85
Q3 mass	204.74
Dwell time (msec)	1300
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	-50
Entrance potential (EP)	-10
Collision energy (CE)	-28
Collision cell exit potential (CXP)	-7
Electron multiplier setting (CEM)	-1800

Period 3. Last for 1.5 min

This period is used to monitor SYN-501406 in a negative mode.

Operating Conditions:

Interface	:	Turbo Ion-Spray
Polarity	:	Negative
Nebuliser Gas (GS1)	:	50
Turbo Gas (GS2)	:	50
Curtain gas (CUR)	:	25 (arbitrary units)

TABLE 1. LC/MS/MS SYSTEM I AND OPERATING CONDITIONS (CONTINUED)

Temperature (TEM)	:	600
Ion-Spray voltage	:	-3500
Collision gas (CAD)	:	Nitrogen 4.0 (arbitrary units)
Scan type	:	MRM

Analyte	SYN-501406
Q1 mass	293.88
Q3 mass	190.85
Dwell time (msec)	600
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	-55
Entrance potential (EP)	-10
Collision energy (CE)	-34
Collision cell exit potential (CXP)	-11
Electron multiplier setting (CEM)	-1800

Period 4. Last for 0.9 min

This period is used to monitor CGA-293343, CGA-322704, and CGA-355190 in a positive mode.

Operating Conditions:

Interface	:	Turbo Ion-Spray
Polarity	:	Positive
Nebuliser Gas (GS1)	:	50
Turbo Gas (GS2)	:	60
Curtain gas (CUR)	:	15 (arbitrary units)
Temperature (TEM)	:	600
Ion-Spray voltage	:	5500
Collision gas (CAD)	:	Nitrogen 3.0 (arbitrary units)
Scan type	:	MRM

TABLE 1. LC/MS/MS SYSTEM I AND OPERATING CONDITIONS (CONTINUED)

Analyte	CG-293343	CGA-322704	CGA-355190
Q1 mass	292.09	250.12	248.12
Q3 mass	211.15	131.85	174.95
Dwell time	150	150	150
Resolution Q1	UNIT	UNIT	UNIT
Resolution Q3	UNIT	UNIT	UNIT
Declustering potential (DP)	41	51	56
Entrance potential (EP)	10	10	10
Collision energy (CE)	17	21	29
Collision cell exit potential (CXP)	18	12	16
Electron multiplier setting (CEM)	1800	1800	1800

Period 5. Last for 2 min

This period is used to monitor NOA-404617 in a positive mode.

Operating Conditions:

Interface	:	Turbo Ion-Spray
Polarity	:	Positive
Nebuliser Gas (GS1)	:	50
Turbo Gas (GS2)	:	60
Curtain gas (CUR)	:	15 (arbitrary units)
Temperature (TEM)	:	600
Ion-Spray voltage	:	5500
Collision gas (CAD)	:	Nitrogen 3.0 (arbitrary units)
Scan type	:	MRM

Analyte	NOA-404617
Q1 mass	237.08
Q3 mass	174.95
Dwell time	400
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	31
Entrance potential (EP)	10
Collision energy (CE)	17
Collision cell exit potential (CXP)	16
Electron multiplier setting (CEM)	1800

TABLE 2. LC/MS/MS SYSTEM II AND OPERATING CONDITIONS

This system is used for the determination of CGA-353042 and NOA-407475.

HPLC Instrumentation:

Perkin-Elmer Series 200 Quaternary Gradient Pump
Perkin-Elmer Series 200 Autosampler
Perkin-Elmer Series 200 Column Heater

Operating Conditions

Column Heater: 40 °C
Injector Volume: 20 µL (20-100 uL depending upon instrument sensitivity)
Mobile Phase Flow Rate: 0.4 mL/min
Column: An Upchurch (A-318) pre-column filter (0.5 µm) is installed between the autosampler and columns to prevent fine particles from the sample blocking the columns.
Zorbax SCX guard column (cat# 820950-904, MAC-MOD Analytical) equipped with a guard column holder (cat# 820582-001, MAC-MOD Analytical)
Zorbax 300-SCX, 5 um 150x2.1mm (cat# 883700-704, MAC-MOD Analytical)

Mobile phase C = 50% acetonitrile/25 mM ammonium acetate.

Total Run Time: 10 min

Analyte Retention Times:	CGA-353042	3.46 min
	NOA-407475	3.30 min

MS Instrumentation:

PE Sciex API-4000 Triple Quadrupole Mass Spectrometer
Instrument Control and Data Collection: Dell Computer, Model Precision 340

Software: Analyst 1.3

All software programs are written and provided by PE Sciex.
Different versions of the system and applications software may be used provided they are able to collect and process the data properly.

TABLE 2. LC/MS/MS SYSTEM II AND OPERATING CONDITIONS (CONTINUED)

Period 1. Last for 2.0 min

This period is a delay period where the ion-spray voltage is set to 0 volt so that none of compounds in the HPLC eluent, (particularly the early eluting polar organic compounds and salts) is charged; therefore, they do not enter the mass spectrometer and keep the instrument clean.

Operating Conditions:

Interface	:	Turbo Ion-Spray
Polarity	:	Positive
Nebuliser Gas (GS1)	:	50
Turbo Gas (GS2)	:	50
Curtain gas (CUR)	:	15 (arbitrary units)
Temperature (TEM)	:	600
Ion-Spray voltage	:	0
Collision gas (CAD)	:	Nitrogen 3.0 (arbitrary units)
Scan type	:	MRM

Period 2. Last for 5.0 min

Operating Conditions:

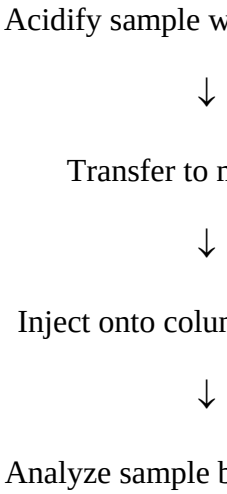
Interface	:	Turbo Ion-Spray
Polarity	:	Positive
Nebuliser Gas (GS1)	:	50
Turbo Gas (GS2)	:	50
Curtain gas (CUR)	:	45 (arbitrary units)
Temperature (TEM)	:	600
Ion-Spray voltage	:	5000
Collision gas (CAD)	:	Nitrogen 3.0 (arbitrary units)
Scan type	:	MRM

LC Flow into the Mass Spectrometer with no split

TABLE 2. LC/MS/MS SYSTEM II AND OPERATING CONDITIONS (CONTINUED)

Analyte	CGA-353042	NOA-407475
Q1 mass	116.09	247.10
Q3 mass	85.95	132.05
Dwell time	2800	2800
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	45	45
Entrance potential (EP)	10	10
Collision energy (CE)	15	35
Collision cell exit potential (CXP)	8	12
Electron multiplier setting (CEM)	1800	1800

FIGURE 2. METHOD 3103-03 FLOW DIAGRAM FOR WATER



14.0 REFERENCES

(1) T.J. Mayer, "Validation of Draft Method 3103-03 for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection," including Protocol Appendix 1.

S:\2003\3103-03\METHOD\DOC\final method 3103-03.doc rg/sbh: 3/18/04