BASF

BASF Agro Research
Residue Sciences Section
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Recommended Method of Analysis - M 3503

BAS 560 F (CL 375839): LC/MS Determinative and LC/MS/MS Confirmatory Method for Determination of BAS 560 F and CL 375816 Residues in Drinking Water and Surface Water

A. PRINCIPLE

Residues of BAS 560 F are extracted from water sample using liquid-liquid partitioning. Residues of CL 375816 are extracted from water sample using liquid-liquid partitioning and SAX solid-phase extraction techniques. Quantitation of BAS 560 F and CL 375816 residues are accomplished by liquid chromatography with mass spectrometric detection. Results are calculated as BAS 560 F and CL 375816 by the direct comparison of the peak responses of the sample to those of external standards. The validated sensitivity (LOQ, Limit of Quantitation) of the method is 0.050 ppb for each compound and the limit of detection (LOD) is 0.010 ppb.

B. SAFETY PRECAUTIONS

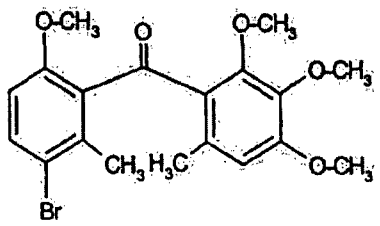
Precautions are to be taken to restrict exposure to all chemicals specified in this method. The proper use of appropriate safety glasses/ goggles, gloves, laboratory coat, ventilation, and handling techniques are to be observed. Appropriate Material Data Handling Sheets are to be reviewed for all chemicals used in the method prior to starting work. The current MSDS information for BAS 560 F and CL 375816 are obtainable from BASF Corporation, BASF Agro Research, P O Box 400, Princeton, New Jersey 08543-0400.

C. REAGENTS

(Items from manufacturers other than those listed may be used if proven to be functionally equivalent.)

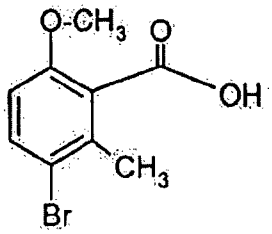
1. Analytical Standards: Analytical grade, known purity, obtained from BASF Corporation, BASF Agro Research, P.O. Box 400, Princeton, New Jersey 08543-0400, USA.

   a. BAS 560 F (CL 375839): 3'-bromo-2,3,4,6'-tetramethoxy-2'6-methyl-methanone



Molecular Formula: $C_{19}H_{21}BrO_5$ M.W. = 409.3

   b. CL 375816: 2-methyl-3-bromo-6-methoxy-benzoic acid



Molecular Formula $C_9H_9BrO_3$ M.W = 245.10

2. High Purity Water: Millipore Milli-Q UV Plus System, Millipore, Inc.

3. Solvents: B & J Brand High Purity Solvents, Baxter, Burdick and Jackson, Muskegon, MI

   a. Methanol
   b. Dichloromethane

4. Chemicals:

   a. Acetic Acid (99.7%), E.M. Sciences, A.C.S. Reagent, Catalog Number AX-0073-6, Gibbstown, NJ.
   b. Formic acid (~98%), Catalog No. 06440, Fluka Chemika, Ronkonkoma, NY.
   c. Ammonium Hydroxide (28-30%), Catalog Number AX-1303-13, EM Sciences, Gibbstown, NJ.
   d. Ammonium Acetate (~100%), E.M. Sciences, A.C.S. Reagent, Catalog Number AX-1220-1, Gibbstown, NJ.

5. Solutions:

   a Solution A (50% Methanol in Water): Add 500 mL of methanol to 500 mL of Milli-Q water and mix well.

   b. Solution B (30% Methanol: 69%Water: 1%Acetic Acid): Add 300 mL of methanol to 690 mL of Milli-Q water, then add 10 mL of acetic acid and mix well.

   c. Solution C (1% Formic Acid in Methanol): Add 1.0 mL of formic acid to 99 mL of methanol and mix well.

   d. Mobile Phase A (0.05% Acetic Acid in Water): Add 0.5 mL of acetic acid to 999.5 mL of Milli-Q water and mix well

   e Mobile Phase B (10 mM Ammonium Acetate in Methanol) Add 0.77 g of ammonium acetate to 1000 mL of methanol and mix well (**Ammonium acetate must be freshly opened**).

D. APPARATUS

(Items from other manufacturers may be used if they are proven to be functionally equivalent)

1. Balance, Analytical: Sartorius R200D Balance, readable to 0.00001 g.

2. Micro weighing-transfer funnels: 15 x 40 mm, Cat. No. 662101, Wheaton, Millville, NJ.

3. Amber Glass Bottles: VWR TraceClean, PC-series, 125-mL capacity, Cat. No. 15900-136, VWR Scientific Products, South Plainfield, NJ.

4. General Laboratory Glassware: Assorted volumetric flasks, volumetric pipettes, mixing cylinder, filtering flasks, evaporation flasks, Buchner funnels and separatory funnels.

5. Rotary Evaporator: Buchler Instruments Model PF-10 DN, or equivalent, equipped with a heated water bath maintained at approximately 35°C in which the evaporation flasks can be partially submerged.

6. Vacuum Sample Processing Station: VacMaster, Cat. No. 121-1010, with PTFE stopcocks, Cat. No. 121-0009, distributed by Jones Chromatography, Lakewood, CO.

7. Solid Phase Extraction Cartridge: ISOLUTE SAX cartridge, 1 g/6 mL, Catalog Number 500-0100-C, Jones Chromatography, Lakewood, CO.

8. Disposable Scintillation Vial: 20 mL, Catalog No. 74516-20, Kimble Glass, Vineland, NJ.

9 Mini-Vap Evaporator/Concentrator For connection to a dry nitrogen source, Supelco Catalog No 2-2971, Supelco, Inc, Bellefonte, PA

10 Sonicator: BRANSONIC Ultrasonic Cleaner®, Model 1210, Branson ULtrasonic Corporation, Danbury, CT.

11. Disposable Pipets: SAMCO Transfer pipets, Catalog Number 202, Samco Scientific Corporation, San Francisco, CA.

12. Autosampler Vials: Amber, with cap, Catalog Number HP-5182-0556, VWR Scientific Products, South Plainfield, NJ.

13. Glass Microfibre Filters: 9.0 cm, GF/B Grade, Catalog Number 28497-492, VWR Scientific Products, South Plainfield, NJ.

14. LC/MS Instrumentation:

    a. Mass Spectrometer: Finnigan MAT LCQ Deca.

    b. LC/MS Interface: Finnigan MAT atmospheric pressure ionization (API) system operated in the APCI mode.

    c. Liquid Chromatograph: Hewlett Packard Series 1100 LC pump and an HP Series 1100 Auto Injector controlled by an HP Series 1100 System Controller.

    d. LC Columns: 10-cm x 4.6-mm ID, 2 µm particle size, TSK-GEL Super-ODS, Catalog Number 18197, TosoHaas, Mongomeryville, PA.

    e. Guard Filter and Holder: Guard Filter Holder, Catalog Number 18206, and Guard Filter, Catalog Number 18207, TosoHaas, Montgomeryville, PA.

E. PREPARATION OF STANDARD SOLUTIONS

All stock, fortification and calibration standard solutions should be stored refrigerated in amber bottles at approximately 4°C and are stable for 2 weeks past the preparation date of the stock solutions Standard solutions should be brought to room temperature prior to use.

1. Stock Standard Solutions:

   a. BAS 560 F: Weigh accurately a known amount (~5.0 mg) of BAS 560 F into a 50-mL volumetric flask. Dilute to the mark with methanol and mix well. Calculate and record the exact concentration after correcting for standard purity.

   b. CL 375816: Weigh accurately a known amount (~5.0 mg) of CL 375816 into a 50-mL volumetric flask. Dilute to the mark with methanol and mix well. Calculate and record the exact concentration after correcting for standard purity.

2. Mixed Fortification Standard Solutions:

   a. 1.0 µg/mL Mixed Fortification Standard Solution: Pipette into a single 100-mL volumetric flask, an appropriate amount of each stock solution to deliver 100 µg of each compound. Dilute to the mark with methanol and mix well.

   b. 0.050 µg/mL Mixed Fortification Standard Solution: Pipette 5.0 mL of the 1.0 µg/mL mixed fortification standard (E.2 a.) into a 100-mL volumetric flask, dilute to the mark with methanol and mix well.

   c. 0.0050 µg/mL Mixed Fortification Standard Solution: Pipette 10 mL of the 0.050 µg/mL mixed fortification standard (E.2.b.) into a 100-mL volumetric flask, dilute to the mark with methanol and mix well.

3. Calibration Standard Solutions of BAS 560 F:

   a. 5.0 ng/mL Calibration Standard Solution: Pipette 0.50 mL of the 1.0 µg/mL mixed fortification standard (E.2.a) into a 100-mL volumetric flask, dilute to the mark with Solution A (C.5 a) and mix well.

   b 2.5 ng/mL Calibration Standard Solution. Pipette 25 mL of the 5 0 ng/mL calibration standard (E.3.a) into a 50-mL volumetric flask, dilute to the mark with Solution A (C.5.a) and mix well.

c. 1.25 ng/mL Calibration Standard Solution: Pipette 25 mL of the 2.5 ng/mL calibration standard (E.3.b.) into a 50-mL volumetric flask, dilute to the mark with Solution A (C.5.a.) and mix well.

Use the 5.0, 2.5 and 1.25 ng/mL calibration standard solutions for the linearity check (see section G). Use the 2.5 ng/mL calibration standard solution as the bracketing standard.

4. Calibration Standard Solutions of CL 375816:

a. 10.0 ng/mL Calibration Standard Solution: Pipette 1.0 mL of the 1.0 µg/mL mixed fortification standard (E.2.a.) into a 100-mL volumetric flask, dilute to the mark with Solution B (C.5.b.) and mix well.

b. 5.0 ng/mL Calibration Standard Solution: Pipette 25 mL of the 10 ng/mL calibration standard (E.4.a) into a 50-mL volumetric flask, dilute to the mark with Solution B (C.5.b.) and mix well.

c. 2.5 ng/mL Calibration Standard Solution: Pipette 25 mL of the 5.0 ng/mL mixed calibration standard (E.4.b.) into a 50-mL volumetric flask, dilute to the mark with Solution B (C.5.b.) and mix well.

Use the 10, 5.0 and 2.5 ng/mL calibration standard solutions for the linearity check (see section G). Use the 5.0 ng/mL calibration standard solution as the bracketing standard.

F. LC/MS CONDITIONS AND SETUP

Operating conditions described below are provided for use as a guide in establishing actual operating conditions. These conditions are specific for the instrument on which they were determined and should be adjusted as necessary to give adequate sensitivity and resolution. Prior to the analysis, the mass spectrometer should be tuned using an appropriate reference material to give proper resolution and peak shape and the data system should be calibrated.

1 LC/MS Instrumentation: (Items from other manufacturers may be used provided they are proven to be functionally equivalent to those listed.)

a. Mass Spectrometer: Finnigan MAT LCQ Deca.

b. LC/MS Interface: Finnigan MAT atmospheric pressure ionization (API) system operated in the APCI mode.

c. Liquid Chromatograph: Hewlett Packard Series 1100 LC pump and an HP Series 1100 Auto Injector controlled by an HP Series 1100 System Controller.

d. LC Columns: 10-cm x 4.6-mm ID, 2 µm particle size, TSK-GEL Super-ODS, Catalog Number 18197, TosoHaas, Mongomeryville, PA.

e. Guard Filter and Holder: Guard Filter Holder, Catalog Number 18206, and Guard Filter, Catalog Number 18207, TosoHaas, Montgomeryville, PA.

2. Mobile Phase:

a. Mobile Phase A: 0.05% Acetic Acid in Water
b. Mobile Phase B: 10 mM Ammonium Acetate in Methanol

3. LC/MS Conditions:

a. HPLC

HPLC Column Temperature: Ambient

HPLC Mobile Phase Gradient for BAS 560 F:

Gradient Time (min)	% Mobile Phase A	% Mobile Phase B
0.0	70	30
6.0	0	100
8.0	0	100
9.0	70	30
10.0	70	30

Post Run Time	0.0 min
Injection Volume	100 µL
Flow Rate	1.0 mL/min
Retention Time	6.2-6.8 min

HPLC Mobile Phase Gradient for CL 375816:

Gradient Time (min)	% Mobile Phase A	% Mobile Phase B
0.0	70	30
5.0	45	55
6.0	0	100
9.0	0	100
10.0	70	30
12.0	70	30

Post Run Time: 0.0 min.
Injection Volume: 100 µL
Flow Rate: 0.75 mL/min.
Retention Time: 4.7-5.2 min.

b. LC/MS Conditions:
Settings For BAS 560F

Divert Valve:	0.0 min to waste, 5.0 min to source, 7.50 min to waste
Ionization Mode:	Positive APCI
Capillary Temperature:	150 °C
Vaporizer Temperature:	400 °C
Sheath Gas Flow:	80 mL/min
Auxiliary Gas Flow:	20 mL/min
Source Voltage:	6 kV
Source Current:	5 uA
Capillary Voltage:	41 V
Tube Lens Offset:	55 V
Octapole 1 Offset:	-7.5 V
Octapole 2 Offset	-9.5 V
InterOctapole Lense.	-18 V
Entrance Lens:	-36 V
Dynode Voltage:	-15 kV
Electronic Multiplier Voltage:	-1022 V
AGC:	On
Injection Waveforms	Off
Scan Range:	MS Full Scan Mode m/z 200'-500' SIM Scan Mode m/z 411', IsoW =1 0

Maximum Ion Injection Time:	200 ms
Microscan:	3 per scan
Settings For CL 375816	
Divert Valve:	0.0 min to waste, 3.5 min to source, 6.5 min to waste
Ionization Mode:	Negative APCI
Capillary Temperature:	150 °C
Vaporizer Temperature:	400 °C
Sheath Gas Flow:	80 mL/min
Auxiliary Gas Flow:	20 mL/min
Source Voltage:	5 kV
Source Current:	80 uA
Capillary Voltage:	-16 V
Tube Lens Offset:	-60 V
Octapole 1 Offset:	8.8 V
Octapole 2 Offset:	11 V
InterOctapole Lense:	26 V
Entrance Lens:	38 V
Dynode Voltage:	15 kV
Electronic Multiplier Voltage:	-1022 V
AGC:	On
Injection Waveforms:	Off
Scan Range:	MS Full Scan Mode: m/z 180-250 SIM Scan Mode: m/z 243, IsoW =1.0
Maximum Ion Injection Time:	200 ms
Microscan:	3 per scan

4 MS Detector Setup:

a. Determine the mass centroids of the (M+H) ions using the conditions detailed in F.3. for BAS 560 F (nominal m/z of 411) by making 100-µL injections of the 1 0 µg/mL prepared as described in section E.2 a but diluted with Solution A (C 5 a.) See Figures 1 for example of the mass centroid determination of BAS 560 F

b. Determine the mass centroids of the (M-H) ions using the conditions detailed in F.3. for CL 375816 (nominal m/z of 243) by making 100-µL injections of the 1.0 µg/mL prepared as described in section E.2.a but diluted with Solution B (C.5.b.). See Figures 6 for example of the mass centroid determination of CL 375816.

c. During data processing, display the (M+H) ions for BAS 560 F and (M-H) ions for CL 375816 with a range of only 1 mass unit.

G. LINEARITY CHECK

The linearity of response of the LC/MS system should be confirmed whenever a new column or instrument is used, following any instrument modification, or if there is a significant alteration of the chromatographic conditions. The linearity of response of each compound should also be confirmed by injecting **duplicate** injections of all three Calibration Standards (E.3. for BAS 560 F and E.4. for CL 375816) prior to injection of each set of samples analyzed.

1 Instrument sensitivity should be set so that a 250 pg injection of BAS 560 F (100 µL of the 2.5 ng/mL Working Standard, E.3.b.) or 500 pg injection of CL 375816 (100 µL of the 5.0 ng/mL Working Standard, E.4.b.) gives a similar signal to noise ratio as shown in Figure 2 (for BAS 560 F) or Figure 7 (for CL 375816). Injections of the Working Standard should be made until a reasonably constant response is obtained.

2. Make 100-µL injections of the 1.25, 2.5 and 5.0 ng/mL Calibration Standards (E.3.) for BAS 560 F or 2.5, 5.0 and 10 ng/mL Calibration Standards (E.4.) for CL 375816.

3. For each compound, determine the response factor (ratio) for all injections by dividing the peak response by the amount (picograms) injected. Calculate the average response factor, the correlation coefficient (r) for the linear relationship between the nominal standard concentrations and the actual response. A deviation of any standard response by more than 20% from the average or a correlation coefficient of <0.99 indicates instrumental difficulties or incorrect standard preparation which must be corrected before proceeding with the analyses.

H. SAMPLE PREPARATION

Allow frozen water samples to thaw completely at room temperature and then mix or shake well to achieve a homogenous sample. Surface water sample should be filtered before use. Filter an adequate amount of water sample through double layers of glass- microfibre filter paper fitting into a 9-cm Buchner funnel placing over the top of a 500-mL filtering flask using a filter support collar.

I. RECOVERY TEST

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. At least one concurrent fortified control sample must be run with each set of six samples analyzed. These fortifications should cover the range of expected residue values. If only a single fortified control sample is run, it should be at the validated sensitivity (LOQ) of the method. The volume of the fortification standard should be kept to a minimum.

1. Accurately measure 100 mL of water into a 500-mL separatory funnel.

2. Add, by pipette, the volume of the Mixed Fortification Standard appropriate to the fortification level to be tested. The fortification volume should be ≤1.0 mL. Suggested volumes of the Mixed Fortification Standards (E.2.) that will yield various levels of fortification in a 100-mL water sample are listed as follows:

Fortification Level (ppb)	Amount of Analyte Required per 100-mL Sample	Concentration of Fortification Standard Used	Volume of Fortification Standard Used
0.050	0.0050 µg/mL	0.0050 µg/mL	1.0 mL
0.50	0.050 µg/mL	0.050 µg/mL	1.0 mL

3. Continue with the sample analysis steps beginning with step J.2.

J. PARTITIONING CLEANUP

1. Using a 100-mL graduated cylinder, accurately measure 100 mL of water sample and transfer into a 500-mL separatory funnel.

2. Add 1 mL of ammonium hydroxide to water sample in the separatory funnel. Add 100 mL of dichloromethane, stopper, shake vigorously for 60 seconds (releasing the pressure formed as necessary) and let the phases completely separate (Note: **Freshly opened ammonium hydroxide must be used**)

3. Draw the lower dichloromethane phase containing BAS 560 F residues into a 250-mL evaporation flask and evaporate to dryness using a rotary evaporator and a heated water bath maintained at approximately 35°C.

4. Add 1.0 mL of methanol to the 250-mL evaporation flask, stopper and sonicate for 15 seconds. Add 1.0 mL of Milli-Q water to the same evaporation flask, cap and shake to mix well. Transfer into an autosampler vial, using a disposable pipette, for LC/MS analysis of BAS 560 F.

5. Add 4 mL of acetic acid to the aqueous phase left in the separatory funnel from step J.3. Add 100 mL of dichloromethane, stopper, shake vigorously for 60 seconds (releasing the pressure formed as necessary) and let the phases completely separate. Draw the lower dichloromethane phase containing CL 375816 residues into a 500-mL evaporation flask.

6. Repeat the partitioning step with another 100-mL aliquot of dichloromethane and draw the lower dichloromethane phase containing CL 375816 residues into the same 500-mL evaporation flask from step J.5. Evaporate to dryness or as dry as possible using a rotary evaporator and a heated water bath maintained at approximately 35°C.

7. Add approximately 5 mL of methanol to the 500-mL evaporation flask and re-evaporate to dryness. Repeat the re-evaporation with another 5-mL aliquot of methanol. **(Note: It is important to completely remove trace amount of acetic acid before solid phase extraction cleanup or the loss of recovery will occur. Repeat this step again if necessary)**

K. SOLID PHASE EXTRACTION (SPE) CLEANUP FOR CL 375816

Note: For the steps involving the solid phase extraction (SPE) cartridge, do not allow the cartridge sorbent to go dry during pre-conditioning, sample loading,

or washing. If the cartridges inadvertently go dry during pre-conditioning, start over with the pre-washing steps.

1. Using a VacMaster sample processing station, prepare a SAX cartridge (1 g/6 mL) by pre-washing with one cartridge volume* of methanol, follow by one cartridge volumes of 50% methanol in water. (*A "cartridge volume" means the cartridge barrel being filled to the top. With the 1 g/6cc cartridge, this volume is approximately 5 mL.)

2. Add 2 mL of methanol to the 500-mL evaporation flask from step J.7., stopper, sonicate for 15 seconds. Add 2 mL of Milli-Q water to the same 500-mL evaporation flask, swirl to mix well and transfer this solution to the cartridge barrel. Using low vacuum if necessary, draw the sample through the cartridge at the rate of approximately 1 drop per second and discard the eluate.

3. Rinse the 500-mL evaporation flask with 2 mL of 50% methanol in water and add to the cartridge. Using low vacuum if necessary, draw the sample through the cartridge at the rate of approximately 1 drop per second and discard the eluate.

4. Wash the cartridge with one cartridge volume of methanol, using low vacuum if necessary, at the rate of 2-3 drops per second. Do not allow the cartridge to run dry.

5. Turn the vacuum off and remove the top of the vacuum processing station. Place a scintillation vial inside the vacuum processing station under the stopcock needle then replace the top of the vacuum processing station.

6. Elute the residues from the SAX cartridge with one cartridge volume of Solution C (C.5.c.), using low vacuum if necessary, at the rate of 1-2 drops per second. Collect the eluate in the scintillation vial. Turn the vacuum off, remove the top of the vacuum processing station and carefully remove the scintillation vial from the vacuum processing station.

7 Evaporate the eluant in the scintillation vial to dryness using the Mini-Vap evaporator/concentrator with a nitrogen stream and temperature setting at about 40°C.

8 Add 1.0 mL of Solution B (C.5.b.) to the scintillation vial, cap tightly and sonicate for 15 seconds. Transfer into an autosampler vial, using a disposable pipette, for LC/MS analysis of CL 375816.

L. LC/MS ANALYSIS

1. Using the parameters detailed in Section F, make 100-µL injections of the 2.5 ng/mL Working Standard (E.3.b.) for the analysis of BAS 560 F or the 5.0 ng/mL Working Standard (E.4.b.) for the analysis of CL 375816 until a reasonably constant response is obtained.

2. Establish the linearity of response as detailed in Section G.

3. An injection of the Working Standard is to be made after at least every two sample injections. Use the average analyte response of the standards injected before and after the sample injection (bracketing standards) for the calculation.

4. If an analyte response exceeds the linear range, dilute an aliquot of the sample to an appropriate volume with Solution A (C.5.a.) for the analysis of BAS 560 F or Solution B (C.5.b.) for the analysis of CL 375816, then take an aliquot of this diluted sample for re-injection.

5. If the response of the Working Standard decreases to an unacceptable level during the analysis, instrumental parameters should be adjusted to restore adequate sensitivity. If such adjustments are made, inject duplicate aliquots of the Working Standard to determine the new response values of the standard.

6. The peak response variation between two bracketing standards must not exceed 20%. If the variation exceeds 20%, instrumental parameters should be adjusted to restore adequate sensitivity. If such adjustments are made, inject duplicate aliquots of the Working Standard to determine the new response values of the standard. The samples which were bracketed when this >20% variation occurred must then be re-injected.

M. CALCULATIONS

Calculate the apparent residues (in ppb) of each compound in the injected samples from the sample peak response and the average peak response of the bracketing standards

$$PPB = \frac{R(SAMP) \times V1 \times V3 \times C(STD) \times V5 \times DF \times 1000}{R(STD) \times V2 \times W \times V4}$$

$$\%\,RECOVERY = \frac{PPB\ FOUND \times 100}{FV \times FC \times 1000/W} = \frac{PPB\ FOUND}{PPB\ ADDED} \times 100$$

Where:

R(SAMP) = Peak response of sample

R(STD) = Average peak response of bracketing standards

W = Weight of the sample in grams (100 mL = 100 grams)

V1 = Total volume of extraction solvent in milliliters (use 1 for calculation)

V2 = Aliquot of extract taken for analysis in milliliters (use 1 for calculation)

V3 = Final volume solution for analysis in milliliters (2 mL for BAS 560 F or 1 mL for CL 375816)

V4 = Volume of sample injected in microliters (100 µL)

V5 = Volume of Working Standard injected in microliters (100 µL)

C(STD) = Concentration of Working Standard injected in µg/mL (2.5 ng/mL = 0 0025 µg/mL for BAS 560 F or 5 0 ng/mL = 0 0050 µg/mL for CL 375816)

DF = Dilution Factor (1 unless further dilution is needed)

FV = Fortification volume in milliliters

FC = Fortification Solution concentration in µg/mL

1000 = A conversion factor from µg to ng

Typical mass spectra for the mass centroid determination from the LC/MS analysis of BAS 560 F are shown in Figures 1. Typical chromatograms for the LC/MS determinative analysis of BAS 560 F in water are shown in Figures 2 through 5.

Typical mass spectra for the mass centroid determination from the LC/MS analysis of CL 375816 are shown in Figures 6. Typical chromatograms for the LC/MS determinative analysis of CL 375816 in water are shown in Figures 7 through 10.

N. LC/MS/MS CONFIRMATORY METHOD CONDITIONS AND SETUP

The LC/MS conditions and setup as detailed in Section F are to be used for the LC/MS/MS confirmatory, qualitative analysis with the following changes:

1. LC/MS/MS Conditions:

a. Settings For BAS 560F

Divert Valve:	0.0 min to waste, 5 0 min to source, 7.50 min to waste
Ionization Mode:	Positive APCI
Capillary Temperature:	150 °C
Vaporizer Temperature:	400 °C
Sheath Gas Flow:	80 mL/min
Auxiliary Gas Flow	20 mL/min
Source Voltage:	6 kV
Source Current.	5 uA
Capillary Voltage	41 V
Tube Lens Offset.	55 V
Octapole 1 Offset	-7.5 V
Octapole 2 Offset	-9.5 V
InterOctapole Lense	-18 V

Entrance Lens:	-36 V
Dynode Voltage:	-15 kV
Electronic Multiplier Voltage:	-1022 V
AGC:	On
Injection Waveforms:	Off
Parent Ion:	m/z 411$^+$, IsoW =1.5
Activation Amplitude:	20%
Q:	0.25
Activation Time:	30 ms
Scan Range:	MS/MS Full Scan Mode: m/z 150$^+$-450$^+$
Maximum Ion Injection Time:	200 ms
Microscan:	3 per scan

b. Settings For CL 375816

Divert Valve:	0.0 min to waste, 3.5 min to source, 6.5 min to waste
Ionization Mode:	Negative APCI
Capillary Temperature:	150 °C
Vaporizer Temperature:	400 °C
Sheath Gas Flow:	80 mL/min
Auxiliary Gas Flow:	20 mL/min
Source Voltage:	5 kV
Source Current:	80 uA
Capillary Voltage:	-16 V
Tube Lens Offset:	-60 V
Octapole 1 Offset:	8.8 V
Octapole 2 Offset:	11 V
InterOctapole Lense:	26 V
Entrance Lens:	38 V
Dynode Voltage:	15 kV
Electronic Multiplier Voltage:	-1022 V
AGC:	On
Injection Waveforms	Off
Parent Ion	m/z 243$^-$, IsoW =1 0
Activation Amplitude	20%
Q:	0.25

Activation Time:	30 ms
Scan Range:	MS/MS Full Scan Mode: m/z 150-250 for mass centroid determination of product ions. m/z 180-220 for sample analysis.
Maximum Ion Injection Time:	200 ms
Microscan:	3 per scan.

2. MS/MS Detector Setup:

   a. For BAS 560 F, change the scan mode to MS/MS. Set the mass centroid of the (M+H) ion of BAS 560 F (nominal m/z of 411) as the parent mass with an isolation width of 1.5 and an activation amplitude of 20%. Set the MS/MS full scan range to m/z 150–450 to monitor for the product ions (nominal m/z of 209.1 and m/z 229.1).

   b. For CL 375816, change the scan mode to MS/MS. Set the mass centroid of the (M-H) ion of CL 375816 (nominal m/z of 243) as the parent mass with an isolation width of 1.0 and an activation amplitude of 20%. Set the MS/MS full scan range to m/z 180-220 to monitor for the product ion (nominal m/z of 198.8).

   c. During data processing, display the full scan mass spectrum of product ions.

O. LC/MS/MS CONFIRMATORY ANALYSIS

In general, one control and three fortified-control samples at LOQ (0.050 ppb) from each type of water sample are qualitatively analyzed for confirmatory analysis.

1. Using the conditions detailed in Section N.1.a. for BAS 560 F or N.1.b. for CL 375816, make a 100-µL injections of the 2.5 ng/mL Working Standard for BAS 560 F or 5.0 ng/mL Working Standard for CL 375816 until a reasonably constant response is achieved

2 For the analyses, make 100-µL injections of the Working Standard and samples, with a Working Standard injection being made after at least every two sample injections.

3. If the response of the Working Standard decreases to an unacceptable level during the analysis, instrumental parameters should be adjusted to restore adequate sensitivity. If such adjustments are made, inject duplicate aliquots of the Working Standard to determine the new response values of the standard.

4. The sample is confirmed as containing of either BAS 560 F or CL 375816 residues when:

   a. The retention time of the presumed analyte in the sample is within 10 seconds of the average retention time of the analyte peak in the bracketing standards.

   b. The spectrum of presumed analytes in the samples correspond to those of bracketing standards.