BASF

BASF Agro Research
Residue Sciences Section
P.O. Box 400
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Recommended Method of Analysis - M 3441

BAS 560 F (CL 375839) Liquid Chromatographic/Mass Spectrometric (LC/MS) Determinative and LC/MS/MS Confirmatory Method for BAS 560 F and CL 377160 Residues in Soil

A. PRINCIPLE

Residues of BAS 560 F (also known as CL 375839) and CL 377160 are extracted from soil sample using triethylamine/water/acetonitrile solution and purified using solid-phase extraction techniques. Quantitation of BAS 560 F and CL 377160 residues are accomplished by liquid chromatography/ atmospheric pressure chemical ionization mass spectrometry The confirmatory analysis involves liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (LC/MS/MS) of the isolated residue. Results are calculated as BAS 560 F and CL 377160 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 5 ppb for each compound

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 coats, 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

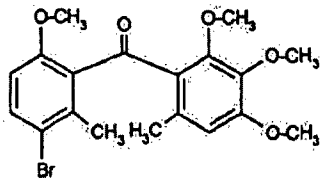
The current MSDS information for BAS 560 F is obtainable from BASF Corporation, BASF Agro Research, P O Box 400, Princeton NJ, 08543-0400

C. REAGENTS

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

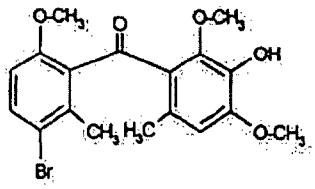
1. Analytical Standards Analytical grade, known purity, obtained from BASF Corporation, BASF Agro Research, P O Box 400, Princeton, New Jersey 08543-0400, USA

   a. BAS 560 F 5-bromo-6,6'-dimethyl-2,2',3',4'-tetramethoxy benzophenone



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

   b. CL 377160 (3-bromo-6-methoxy-2-methylphenyl)(3'-hydroxy-2',4'-dimethoxy-6'-methylphenyl)-methanone



   Structural Formula $C_{18}H_{19}BrO_5$ M.W. 395.3

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 Acetonitrile
b Methanol
c. Methylene Chloride

4 Chemicals:

a Acetic Acid (99.7%), "Baker Analyzed" Reagent, A.C.S. Reagent, Cat. No. 9508
b. Triethylamine (98%), EM Science, Cat. No. TX1200-5

5 Solutions:

a Extraction Solvent: Add 0.5 mL of Triethylamine to 90 mL of acetonitrile and 9.5 mL of Milli-Q water. Mix well.

b 0.5% Acetic Acid in Water: Add 0.5 mL of acetic acid to 99.5 mL of Milli-Q water and mix well

c 10% Methanol in Methylene Chloride: Add 10 mL of methanol to 90 mL of methylene chloride and mix well

d 50% Methanol in Water: Add 50 mL of methanol to 50 mL of Milli-Q water and mix well

e. Mobile Phase A (1% Acetic Acid in Water): Add 1 mL of acetic acid to 99 mL of Milli-Q water and mix well

f Mobile Phase B (1% Acetic Acid in Methanol): Add 1 mL of acetic acid to 99 mL of methanol and mix well

D. APPARATUS

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

1 Assorted Glassware. General laboratory

2 Balance
a Analytical, Sartorius, Model R200D, precision ± 0.05 mg
b Top-Loading, Sartorius, Model 610, precision ± 5.0 mg

3 Liquid Chromatograph/Mass Selective Detector Hewlett Packard 1100 Series MSD, Palo Alto, CA.

4 High Performance Liquid Chromatograph/Mass Spectrometric System: Finnegan-MAT LCQ Deca operated in the APCI ion mode and interfaced through a Finnegan-MAT Atmospheric Pressure Ionization (API) system to the effluent from Hewlett Packard Series 1100 LC pump and an HP Series 1100 Auto Injector controlled by an HP Series 1100 System Controller.

5 HPLC Column: 5-cm x 4.6-mm ID, 2 µm particle size, TSK-GEL Super-ODS, Catalog Number 18154, TosoHaas, Montgomeryville, PA

6 Solid Phase Extraction Cartridge Varian Bond Elut C18 (1 g/6 mL) Part. No. 1225-6001 Varian, Harbor City, CA.

7. Solid Phase Extraction Cartridge Adapter IST Isolute PTFE Column Adapters, Cat No. 120-1100, International Sorbent Technology, Mid Glamorgan, UK

8 Sample Processing Station IST VacMaster equipped with a PTFE stopcock/needle assembly, Cat. No 121-1010, International Sorbent Technology, Mid Glamorgan, UK

9 Disposable Reservoirs: 25 mL, IST Cat No 120-1007-E.

10 Disposable Scintillation Vial. 20 mL, Cat. No 74516-20, Kimble Glass, Vineland, NJ.

11 Mini-Vap Evaporator/Concentrator: For connection to a dry nitrogen source, Supelco Cat. No 2-2971, Supelco, Inc, Bellefonte, PA.

12 Microwave Solvent Extraction System: Model MES-1000, CEM Corp, Matthews, NC.

13. Microwave Extraction Vessel Assemblies Set of six assemblies, Part Number 323033, CEM Corp, Matthews, NC.

14. Vortex Mixer: S/P Vortex Mixer, Catalog Number S8223-1, American Scientific Products, Edison, NJ

15. Sample Vial: 2-mL, clear, Target DP with cap, Part No. 5182-0864, Hewlett Packard, Palo Alto, CA.

16 Pasteur Pipette 5 ¾-in. VWR Brand disposable pipette, Cat. No. 14672-400, VWR, Inc., Bridgeport, NJ.

17. Millex-HV Filter Unit 0.45 µm, Cat. No SLHV025LS, Millipore Products Division, Bedford, MA

18 Disposable Syringe Luer-Lok, 10cc, Cat. No 309604, Becton Dickinson & Company, Franklin Lakes, NJ

E. PREPARATION OF STANDARD SOLUTIONS

All of the standard solutions listed in this section should be stored in amber bottles at approximately 4°C and are stable for 1 month.

1 Stock Solutions

a BAS 560 F (CL 375839): Weigh accurately a known amount (~10 mg) of BAS 560 F into a 100-mL volumetric flask. Dilute to the mark with acetonitrile and mix well. Calculate and record the exact concentration after correcting for standard purity

b CL 377160 Weigh accurately a known amount (~10 mg) of CL 377160 into a 100-mL volumetric flask Dilute to the mark with acetonitrile and mix well Calculate and record the exact concentration after correcting for standard purity

2 Mixed Fortification Standard Solutions

a Mixed Fortification Standard Solution (2.5 µg/mL): Pipette into a single 100-mL volumetric flask, an appropriate amount of each stock solution to deliver 250 µg of each compound. Dilute to the mark with acetonitrile and mix well

b Mixed Fortification Standard Solution (0.25 µg/mL): Pipette 10 mL of the 2.5 µg/mL mixed fortification standard (E.2.a.) into a 100-mL volumetric flask, dilute to the mark with acetonitrile and mix well.

3 Mixed Calibration Standard Solutions

a Mixed Calibration Standard Solution (0 010 µg/mL) Pipette 2.0 mL of the 0 25 µg/mL mixed fortification standard (E 2 b) into a 50-mL volumetric flask, dilute to the mark with 50% methanol in water (solution C.5 d) and mix well

b. Mixed Calibration Standard Solution (0.005 µg/mL): Pipette 25 mL of the 0.010 µg/mL mixed calibration standard (E.3.a) into a 50-mL volumetric flask, dilute to the mark with 50% methanol in water (solution C.5 d.) and mix well

c Mixed Calibration Standard Solution (0.0025 µg/mL): Pipette 25 mL of the 0.005 µg/mL mixed calibration standard (E.3.b.) into a 50-mL volumetric flask, dilute to the mark with 50% methanol in water (solution C.5.d.) and mix well.

d. Mixed Calibration Standard Solution (0.00125 µg/mL) Pipette 25 mL of the 0.0025 µg/mL mixed calibration standard (E.3 c) into a 50-mL volumetric flask, dilute to the mark with 50% methanol in water (solution C 5 d) and mix well

e Mixed Calibration Standard Solution (0.000625 µg/mL) Pipette 25 mL of the 0.00125 µg/mL mixed calibration standard (E 3 d) into a 50-mL volumetric flask, dilute to the mark with 50% methanol in water (solution C 5 d.) and mix well

Use the 0.010, 0.005, 0.0025, 0.00125 and 0.000625 µg/mL mixed calibration standard solutions for the linearity check (see section F). Use the 0.0025 µg/mL mixed calibration standard solution as the bracketing standard

F. LC/MS CONDITIONS

The following conditions are specific for the instruments on which they were determined. Conditions will vary from instrument to instrument and should be adjusted to give sensitivity and adequate resolution. Prior to analysis, the mass spectrometer should be tuned to give proper resolution and peak shape on an appropriate reference material and the data system should be calibrated

1 Mass Spectrometer: Hewlett Packard 1100 Series MSD. LC/MS conditions for analysis of BAS 560 F (CL 375839) and CL 377160 in soil

a HPLC/MS Interface: Atmospheric pressure ionization system operated in the APCI mode

b. High Performance Liquid Chromatograph: Hewlett Packard 1100 Series HPLC equipped with degasser, binary pump, auto sampler, and column compartment

c HPLC Column: 5-cm x 4.6-mm ID, 2 µm particle size, TSK-GEL Super-ODS, Catalog Number 18154, TosoHaas, Montgomeryville, PA.

2 HPLC Conditions for Analysis of BAS 560 F and CL 377160

a	Mobile Phase	A: 1.0% acetic acid in water B: 1.0% acetic acid in methanol
b	Column Temperature	Ambient
c	Flow Rate	1.0 mL/min.
d	Stop Time	15.00 min
e	Post time	4.00 min
f	Injection Volume:	100 µL

g Mobile Phase Gradient (Also, see note to the method in Section O.)

Gradient Time	% Mobile Phase A	% Mobile Phase B
0	60	40
13	20	80
14	20	80
15	60	40

3 MS Conditions for analysis of BAS 560 F and CL 377160 in soil

a.	MSD	Enabled
b	Ionization Mode	APCI
c.	Polarity	Positive
d	Stop Time	14 min.
e	Peak Width	0.09 min.
f	Scan Speed Override	Disabled
g	Fragmentor Ramp	Disabled
h	Time Filter	Disabled
i	Mode	SIM

j SIM Parameters (Also, see note to the method in Section O.)

Time (min)	SIM Ion	Gain	Fragmentor	SIM Resolution	Actual Dwell
6.00	395 397	8.00	65	Low	259
10.50	409 411	8.00	65	Low	259

k	Approx Retention Times	CL 377160 8.6 min. BAS 560 F: 10.8 min.
l	Gas Temp	300°C
m	Vaporizer	300°C
n	Drying Gas	3.0 liter/min
o	Nebulizer Pressure	60 psig
o	Capillary Voltage	4000 V
p	Corona	8.0 uA

G. LINEARITY CHECK

The liquid chromatograph should be checked for linearity of response whenever a new column is used, on each day of use during the analysis of samples from every field residue study, and when the LC/MS system has been adjusted or serviced

1 Inject 100-µL aliquots of the 0.010, 0.005, 0.0025, 0.00125 and 0.000625 µg/mL mixed calibration standard solutions

2. Determine the response factor (ratio) for all injections by dividing the peak response by the amount injected. Calculate the average response ratio A deviation of any standard response factor by more than 15% from the average or a correlation coefficient <0.99 indicates instrumental or standard difficulties, which must be corrected before proceeding with the analyses.

H. SAMPLE PREPARATION

1 Keep all samples frozen until ready for analysis

2 Allow the frozen soil samples to thaw completely in an air-tight container just prior to extraction

3. Thoroughly mix the thawed samples to obtain a homogeneous sample

I. RECOVERY TEST

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. As a quality control measure, at least one control and one concurrent recovery should be run with each set of samples

1 Weigh a 5-g sample of control soil into a microwave extraction vessel

2. Accurately add a volume of mixed standard fortification solution appropriate to the fortification level to be tested. Note: **Use no more than 0.1 mL of fortification solution.**

Example

Fortification Level (ppb)	µg Required per 5 gram Sample	Mixed Fort Standard Solution Used (µg/mL)	Vol. of Soln Used (mL)
5 0	0 025	0 25	0 1
50	0 25	2 5	0 1

3 Continue with the extraction and cleanup steps as described in Section J

J. EXTRACTION

1. Weigh 5 g of soil sample into a microwave extraction vessel

2. Add 25 mL of extraction solvent (C.5.a.) to the soil and stir with a spatula to mix. Place the extraction vessel in the microwave extraction system. Use the following conditions for extraction

a	Power	20% (for 2 samples or fewer) 40% (for 4 samples) 60% (for 6 samples)
b	PSI	50
c	Time to Parameter	10 min.
d	TAP (Time at Parameter)	3 min.
e	Temperature	125°C

Note: The microwave solvent extraction system is extremely sensitive to solvent vapor and will be temporarily disabled if vapors are detected. The rupture disk in the extraction vessel top must not be torn and all connections must be tight before starting the extraction

3. After the extraction is complete, open the microwave door and let the extraction vessels cool to about 45°C before disconnecting and removing the vessels from the microwave

4 After being removed from the microwave, allow the extraction vessels to reach approximately room temperature. Gently uncap each extraction vessel so as not to disturb the soil bed and carefully transfer a 5-mL aliquot of the extract into a 50-mL beaker

5 Add 5 mL of 0.5% acetic acid in Milli-Q water (C.5.b.) to the extract in the beaker and swirl to mix.

K. SOLID PHASE EXTRACTION (SPE)

1 Using a VacMaster sample processing station, prepare a C18 cartridge (1 g/6 mL) by washing with one column volume each, in this order, of methylene chloride, methanol and Milli-Q water. (Do not allow the SPE column to go dry during any of the loading or washing steps)

2 Using an adapter, attach a 25-mL non-fritted reservoir on top of the C18 cartridge. Add the solution from Step J.5 to the reservoir and load the sample onto the C18 cartridge at a flow rate of approximately 2-3 drops per second. Discard the eluate Remove the reservoir and adapter

3. Fill the cartridge with Milli-Q water and pass the water rinse through the cartridge. Once the rinsing is completed, turn the vacuum to the maximum for 1 minute to dry the cartridge.

4. Elute the C18 cartridge with 5 mLs of 10% methanol in methylene chloride (solution C.5.c.), collecting the eluant in a scintillation vial. Evaporate the eluant to dryness using the Mini-Vap evaporator/concentrator with a nitrogen stream and temperature setting at about 60°C. Remove the vial as soon as it dries and add another 10 mLs of 10% methanol and evaporate eluant to dryness again. Remove the vial as soon as it dries.

5. <u>Immediately</u> add 2 mL of methanol to the scintillation vial, cap tightly and vortex for 10 seconds Place the scintillation vial back on the heating surface (60°C) of the Mini-Vap evaporator/concentrator for 15 minutes, vortex for 10 seconds and let cool to room temperature This procedure is required to ensure that the residues are completely dissolved

6 Add 2 mL of Milli-Q water to the vial, cap and vortex for 10 seconds (Note If the solution is cloudy or appears to have particles that are not completely dissolved, filter the solution through a Millex-HV filter unit attached to a disposable syringe into a new scintillation vial). Transfer an aliquot into a HPLC vial for LC/MS analysis.

L. LC/MS ANALYSIS

1 After obtaining a stable chromatographic response as specified in Section G, inject in sequence a 100-µL aliquot of the working standard (0.0025 µg/mL), 100-µL aliquot of a maximum of two samples and another 100-µL aliquot of the working standard.

2 Compare the peak response or area of the sample with those of the 0.0025 µg/mL standards injected before and after the sample (bracketing standards)

3 The variation between the responses of bracketing standards must not exceed 15% from the average of the two responses If the variation exceeds 15%, instrumental parameters should be adjusted to restore instrument performance

4 The variation between the retention times of the bracketing standards must not exceed 5% If the variation exceeds 5%, instrumental parameters should be adjusted to restore instrument performance

5 If a sample peak goes out of the linearity range established with the calibration standards, dilute an aliquot of the sample to an appropriate volume with 50% methanol in water (solution C.5.d.) and re-inject the sample.

6 Samples which appear to have a positive response of ≥5 ppb by LC/MS analysis and requiring LC/MS/MS confirmation are directly amenable to LC/MS/MS analysis (See Section N)

M. CALCULATIONS

For each sample calculation, use the sample peak area and the average peak area measurement of the working standard obtained before and after the sample injections as follows

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

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

Where

R(SAMP) = Peak area of sample

R(STD) = Average peak area of bracketing, working standard

C(STD) = Concentration of working standard (0.0025 µg/mL)

V1 = Volume of extraction solvent in milliliters (25 mL)

V2 = Aliquot of extract taken for analysis in milliliters (5.0 mL)

V3 = Final volume of sample solution for analysis (4.0 mL)

V4 = Volume of sample solution injected (100 µL)

V5 = Volume of standard solution injected (100 µL)

W = Sample weight (5.0 g)

DF = Dilution factor

FV = Fortification volume in milliliters

FC = Fortification concentration (of standard solution added) in micrograms per milliliter

Typical mass chromatograms for soil are shown in Figures 1 through 4

N. LC/MS/MS CONFIRMATORY ANALYSIS

1 Sample Preparation for LC/MS/MS Confirmation: Samples (L.6) that show an individual residue of greater than 5.0 ppb and that require mass spectrometric confirmation are injected directly without any further dilution.

2 LC/MS/MS Standard Solution: Use the 0.0025 µg/mL (2.5 ng/mL) standard (E.3.c.)

3 LC/MS/MS Instrumentation: (Items from other manufacturers may be used if they are proven to be functionally equivalent.)

a Mass Spectrometer: Finnigan MAT LCQ Deca

b. LC/MS/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. Column: 5-cm x 4.6-mm ID, 2 µm particle size, TSK-GEL Super-ODS.

4 LC/MS/MS Condition:

a	LC Column Temperature:	Ambient
b.	Mobile Phases:	A. 1% acetic acid in water B. 1% acetic acid in methanol

c LC Gradient

Time (min)	%A	%B	Flow Rate
0.0	60	40	1.0 mL/min
13.0	20	80	1.0 mL/min
14.0	20	80	1.0 mL/min
15.0	60	40	1.0 mL/min

d	Injection Volume	100 µL
e	Capillary Temperature	250°C
f	Capillary Voltage	30.7 V
g	Polarity	Positive
h	Tube Lens Voltage	20 V
i	Octapole 1 Offset Voltage	-2.6 V
	Octapole 2 Offset Voltage	-6.2 V
j	Interoctopole Lens	-10 V
k	Nitrogen Sheath Gas	80
l	Nitrogen Auxiliary Gas	20
m	Conversion Dynode Voltage	-15 kV
n	Electron Multiplier Voltage	-1120 V
o	Maximum Ion Time	1000 ms
		Full Scan to locate centroids
p	Full Scan Range	m/z 115 to 500
q	Scan Event	MS/MS
r.	Amplitude	20%
s	Segment 1 Duration	10.00 min
	Segment 2 Duration	1.75 min
	Segment 3 Duration	2.25 min
t	Transition Monitored	Segment 1 m/z 396* → 195*
		Segment 2: m/z 410* → 209*
u.	Scan Width	+/-4 mass units
v	High Vacuum	1.5 E-05t
w.	Scan Time	1.0 sec.
x	Approx. Retention Time	CL 377160: 8.5 min
		BAS 560 F: 10.8 min

The conditions described are specific for the instrument on which they were determined. Conditions will vary from instrument to instrument and should be adjusted to give sensitivity and adequate resolution. Prior to analysis, the mass spectrometer should be tuned to give proper resolution and peak shape on an appropriate reference material and the data system should be calibrated.

5 Data Treatment: The sample is confirmed as containing >5.0 ppb of either BAS 560 F or CL 377160 residues when:

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

b The calculated value for each analyte peak in the sample exceeds 5.0 ppb.

Typical mass chromatograms for the working standard, control soil and fortified soil samples are shown in Figures 5-7.

O. NOTE TO THE METHOD

The following mobile phase gradient should be used if there is an unacceptable drift in the baseline and/or the fluctuation in the standard peak responses.

Gradient Time	% Mobile Phase A	% Mobile Phase B
0	50	50
13	10	90
16	10	90
17	50	50

The SIM parameter time must be adjusted accordingly.