SUMMARY

An independent laboratory validation of the method "The Determination of BAS 500 F and Its Metabolites BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7 in Soil Using LC-MS" (Analytical Methods D9812 and D9812/1, modification Change No. 4, Amendment C98016-1), provided by BASF, was conducted. This study was conducted in accordance with U.S. Environmental Protection Agency (EPA) Federal Insecticide, Fungicide and Rodenticide Act Final Rule (40 CFR Part 160), EPA Pesticide Assessment Guidelines, Subdivision O, and US EPA OPPTS 850.7100. The laboratory method validation for the measurement of BAS 500 F and metabolites BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7 in soil was conducted by extracting a 50 g soil sample twice with acetonitrile and diluting with water containing 0.1% formic acid and 4mM ammonium formate for HPLC-MS/MS determination of BAS 500 F, BF 500-3, BF 500-4, BF 500-6, and BF 500-7. The soil marc was re-extracted with 0.1 N sodium hydroxide, acidified, and partitioned with ethyl acetate. An aliquot of the combined extract from the acetonitrile and sodium hydroxide extract was evaporated to dryness and the residue was dissolved in (70:30, v/v) acetonitrile-water containing 0.1% formic acid and 4mM ammonium formate for the HPLC-MS analysis of BF 500-5.

The method was successfully validated. The average recoveries obtained for BAS 500 F, BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7 from fortified samples were within the specified 70-120% range. The relative standard deviations were less than 20% at each spike level. No significant interferences were observed in the control extracts. The method was validated at the LOQ (0.01 mg/kg) and 10xLOQ (0.10 mg/kg) on the first trial.

1.0 PURPOSE

The purpose of this study was to demonstrate that BASF Analytical Method D9812 or D9812/1 "The Determination of BAS 500 F and Its Metabolites BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7 in Soil Using LC-MS" (Modification Change No. 4, Amendment C98016-1) could be performed successfully at an outside facility with no prior experience with the method.

2.0 METHOD SUMMARY

The validation procedure followed the Analytical Method D9812 and D9812/1 "The Determination of BAS 500 F and Its Metabolites BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7 in Soil Using LC-MS", with modification (Change No. 4, Amendment C98016-1) provided by BASF except for a 7 to 10 mL dilution from the combined extract (35 mL) instead of the acetonitrile extract (150 mL). Additional modifications necessary for LC-MS analysis can be found in Sections 3.4 and 3.6. The test substances were extracted from fortified soil by shaking twice with acetonitrile. The soil marc was re-extracted once with 0.1N sodium hydroxide. The extracts in acetonitrile and in 0.1 N sodium hydroxide were collected separately. The alkaline extract was acidified to pH ~2 and a portion of this extract (10 mL) was extracted twice with ethyl acetate. The combined ethyl acetate layer was evaporated to dryness. The residue was

dissolved in 5 mL of acetonitrile. A 30 mL aliquot of the original acetonitrile extract was added the 5 mL. A 10-mL aliquot of the combined extract from the acetonitrile and sodium hydroxide extract was evaporated to dryness. The residue was dissolved in 2 mL (70:30, v/v) of acetonitrile-water containing 0.1% formic acid and 4mM ammonium formate for the HPLC-MS analysis of BF 500-5. A separate 7 mL aliquot of the combined extract from the acetonitrile and sodium hydroxide extract was diluted to 10 mL with water containing 0.1% formic acid and 4mM ammonium formate for HPLC-MS/MS determination of BAS 500 F, BF 500-3, BF 500-4, BF 500-6, and BF 500-7. A method flow chart can be found in Figure 1.

The concentrations of the analytes in the sample extract were determined using the linear calibration curve which was generated based on the analyte peak area versus the analyte concentration in the external standard. Fisons Mass Lynx and Microsoft Excel 97 were used for calculations.

3.0 MATERIALS AND METHODS

3.1 Test/Reference Substances

The test/reference substances for this study were BAS 500 F, BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7. The substances were received frozen from BASF Agricultural Products Center, Research Triangle Park, NC on May 19, 1999. Information regarding the test substances is given in the table below:

BASF Code	BAS 500 F	BF 500-3	BF 500-4	BF 500-5	BF 500-6	BF 500-7
Lot Number	00937-128	01311-150	01183-26	00937-275	01185-025	01185-022
Purity	99.8%	100.0%	99.3%	99.9%	99.8%	99.9%
Expiration Date	07/2000	08/20/2000	11/25/1999	06/04/1999 Expired test material	09/24/1999	09/23/1999
Description	Off White Crystal	Off White to Pale Yellow Crystalline Powder	White Powder	Pale Tan Crystalline Powder	Yellow Powder	Orange Powder
Amount Received	214.5 mg	213.8 mg	191.1 mg	395.3 mg	273.1 mg	251.6 mg
Storage Conditions	Refrigerated	Refrigerated	Refrigerated	Refrigerated	Room Temperature	Room Temperature

Documentation of the synthesis, as well as chemical and physical characterization, of the test/reference substances is maintained by the Sponsor.

Copies of the Certificates of Analysis for the test and reference substances can be found in Appendix I.

3.2 Test System

The test system was comprised of homogenized control soil, BASF sample code RCN 98087, Sample number 46. The BASF RSN is 98087-0046. This soil was chosen to represent a more difficult matrix in comparison with other soils. The homogenized control soil was received frozen from BASF Corporation, Research Triangle Park, North Carolina on July 13, 1999 and was stored at ≤-15°C. The soil was assigned a unique Battelle identifier, 9924-10-01.

3.3 Analytical Method

The recovery data for the study were generated using BASF Analytical Method D9812/1 "The Determination of BAS 500 F, BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7 in Soil Using LC-MS" with modification (Change No. 4, Amendment C98016-1). A method trial sample set consisted of a reagent blank, two unspiked matrix control samples, 5 matrix control samples fortified at LOQ (0.01 mg/kg) and 5 matrix control samples fortified at 10x LOQ (0.10 mg/kg). A total of thirteen samples were processed for a method validation trial. A volumetric pipette was used to administer a standard solution of test substances to the test system.

3.4 Modifications/Observations for the Method

The following is a list of observations noted and modifications made while validating the extraction method.

3.4.1 Change 2, Section 3.3. The F1 (0.7 dilution factor) aliquot was inadvertently taken from the combined 35 mL (Change 4, Section 3.2.4) instead of from the 150 mL acetonitrile extract (3.2.3). This was corrected for in the calculation.

3.4.2 Change 2, Section 3.5. The LC-MS/MS conditions differ from the original method due to different instrument parameters available (i.e., No Turbospray). A Fisons Quattro II was used instead of a PE Sciex API 3000 Biomolecular Mass Analyzer. Modifications to the instrumental analysis conditions were made to accommodate the different LC-MS/MS instrument used. The analysis conditions are detailed in Section 3.6 below.

3.4.3 Change 2, Section 4.2. The calculation for BF 500-5 should include an F3 dilution factor of 0.2. Only 10 mL of the 50 mL of the sodium hydroxide extract is used in the ethyl acetate extraction. Only 30 mL of the 150 mL of the acetonitrile extract is carried through for BF 500-5 analysis.

3.5 Standard Substances and Solutions

Standard solutions for fortifications and LC-MS/MS determination were prepared according to method D9812 Sections 2.1 with modifications in the typical standard concentrations listed in Change 2. Solvent mix solutions were prepared according to method D9812/1 Section 2.3.2 with modifications in mobile phase preparation as listed in Change 2.

3.6 LC-MS/MS System

The following conditions, based on Section 3.5 of the supplied method, were used for LC-MS and LC-MS/MS analysis of BAS 500 F, BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7.

HPLC:	Hewlett Packard (HP) 1100
Analytical Column:	Hewlett Packard, Zorbax SB-C8, 2.1 x 30 mm, 3.5 µm, part # 873700-906
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate
Mobile Phase B:	Methanol with 0.1% formic acid and 4mM ammonium formate
Isocratic Mobile Phase:	80% B + 20% A (All compounds except BF 500-5) Run every 7 minutes.
Gradient Mobile Phase (BF 500-5)	Time (min.) Composition 0.0 50%A + 50%B 3.0 50%A + 50%B 3.1 30%A + 70%B 5.0 30%A + 70%B 5.1 50%A + 50%B 7.0 50%A + 50%B Run every 15 minutes
Flow Rate:	300 µL/minute
Injection Volume:	10 µL

Data Acquisition:	Fisons, MassLynx, v.3.2
Retention Times:	BAS 500 F: ~0.52 minutes, BF 500-3: ~0.52 minutes, BF 500-4: ~ 0.45 minutes, BF 500-5: ~7.58 minutes BF 500-6: ~1.66 minutes, BF 500-7: ~1.40 minutes (A-Isomer) BF 500-7: ~2.58 minutes (B-Isomer)
Mass Spectrometer:	VG/Fisons, Quattro II
Ionization:	Electrospray, Positive Ion Mode (ESP+)
Ions Monitored:	m/z 388→163 (BAS 500 F), m/z 358→132 (BF 500-3) m/z 300→106 (BF 500-4), m/z 195 MS Only (BF 500-5), m/z 611→417 (BF 500-6), m/z 595→207 (BF 500-7)
Source Temperature:	120°C
Drying Gas:	Nitrogen at ~300L/hour
Cone Voltage:	~25 V (All except BF 500-5), ~38 V (BF 500-5)
Collision Gas:	Argon at ~ 2.4 x 10-3 mb (gas cell pressure)
Collision Energy:	~ 25 eV (All except BF 500-5)
Multipliers:	650 V

3.7 Data Calculations/Statistical Methods

The concentrations of BAS 500 F, BF 500-3, BF 500-4, BF 500-6, and BF 500-7 in the fortified samples were determined by using a standard curve obtained from the peak areas of four standard concentrations, a minimum of two injections per concentration. The concentration of BF 500-5 in the fortified samples was determined by using a standard curve obtained from the peak areas of three standard concentrations, three injections per concentration. A standard curve was analyzed before and throughout each sample set. The standard curve for each analyte can be found in Appendix II.

The calibration curve for each analyte was constructed by plotting the areas of each standard versus the amount in pg/µL of standard injected. For BF 500-7, the total area is obtained by adding the area of the A- and B-Isomers. The following equation was used to calculate the concentration of each analyte in the samples:

$$Wi = \frac{R - b}{m}$$

where:

Wi = conc. of analyte in injected aliquot (pg/µL)

R = Response

b = intercept of calibration curve

m = slope of calibration curve.

The mg injected was calculated as follows for all compounds except for BF 500-5:

$$\text{mg injected} = \frac{\text{Sample Weight (50 g) extracted}}{\text{Final volume of extract in acetonitrile (150 mL)}} \times \frac{\text{Aliquot taken from final volume in acetonitrile (30 mL)}}{\text{Final volume of combined extract (35 mL)}} \times 10\ \mu\text{L} \times \frac{\text{Aliquot taken from combined extract in acetonitrile (7 mL)}}{\text{Dilution volume (10 mL)}}$$

The mg injected for BF 500-5 was calculated as follows:

$$\text{mg injected} = \frac{\text{Sample Weight (50 g) extracted}}{\text{Final volume of extract in sodium hydroxide (50 mL)}} \times \frac{\text{Aliquot taken from final volume of sodium hydroxide (10 mL)}}{\text{final volume of combined extract (35 mL)}} \times 10\ \mu\text{L} \times \frac{\text{Aliquot taken from combined extract in acetonitrile (10 mL)}}{\text{Final Volume (2 mL)}}$$

The method recovery was calculated by dividing the determined concentration in ng by the expected concentration of the analyte in the sample and multiplying that ratio by 100. The following formula was used:

$$\text{Method Recovery \%} = \frac{\text{Analyte (determined)}}{\text{Analyte (expected)}} \times 100$$

For example, the concentration of BAS 500 F in soil for the first replicate of the 10x LOQ (0.10 mg/kg) fortification level was calculated as follows:

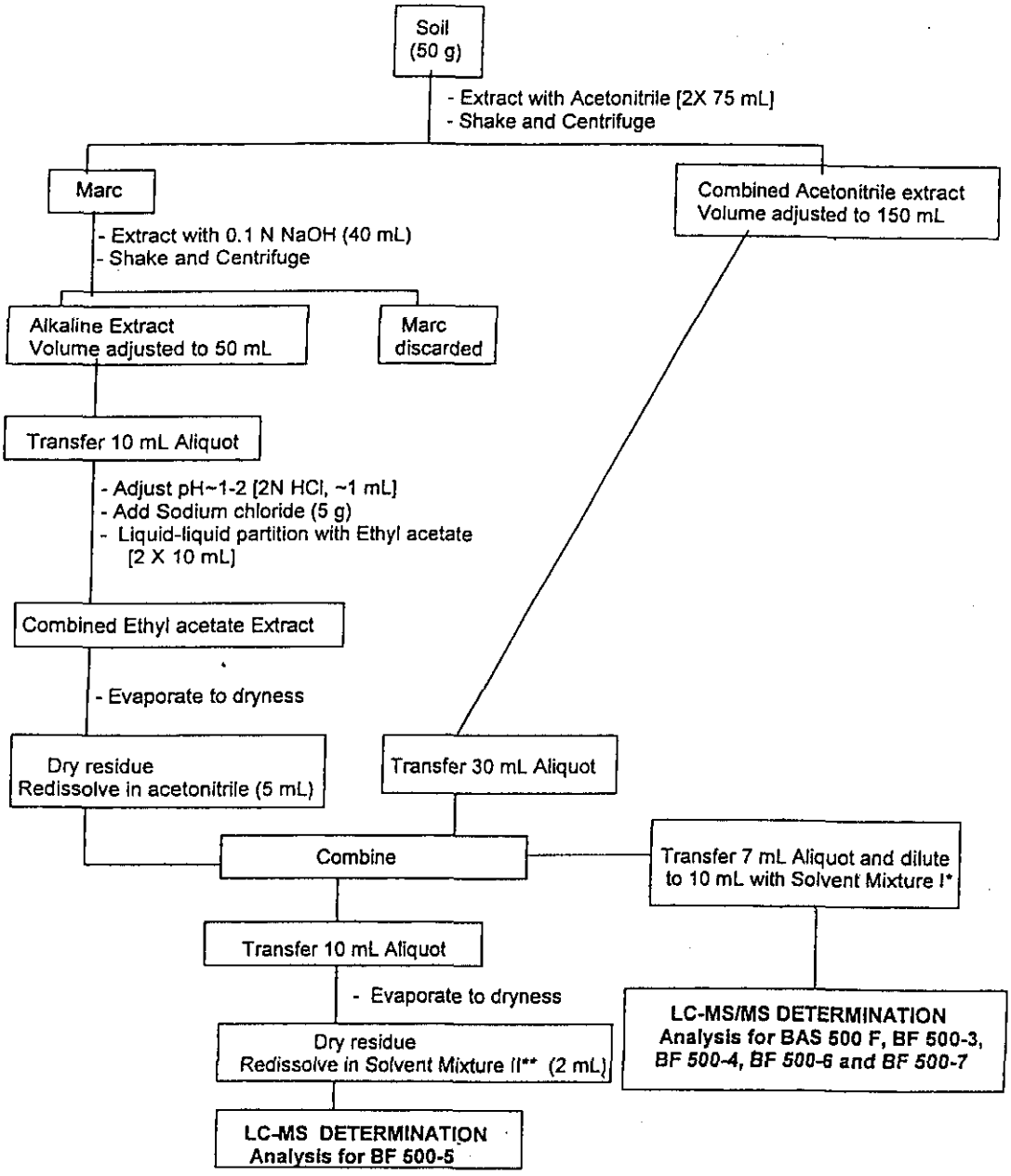
$$\text{Conc. of BAS 500 F (pg / } \mu\text{L)} = \frac{162 - 11.3771}{83.0087} = 1.82 \text{ pg / } \mu\text{L}$$

$$\text{Amount BAS 500 F Injected (ng)} = \frac{1.82 \text{ pg / } \mu\text{L}}{1000 \text{ pg / ng}} \times 10 \ \mu\text{L Injected} = 0.0182 \text{ ng}$$

$$\text{ppm BAS 500 F Injected (ng / mg)} = \frac{0.0182 \text{ ng}}{0.2 \text{ mg Injected}} = 0.0910 \text{ ng / mg (ppm)}$$

$$\text{Method Recovery (\%)} = \frac{0.091 \text{ ppm Found}}{0.100 \text{ ppm Expected}} \times 100 = 91\%$$

FIGURE 1. FLOWCHART



*Solvent Mixture I: Water with 0.1 % formic acid and 4 mM ammonium formate
**Solvent Mixture II: Acetonitrile-water (70:30, v/v) containing 0.1 % formic acid and 4 mM ammonium formate