Study Title

Technical Procedure:
Method for Determination of BAS 500 F, BF 500-3, and BAS 510 F Residues in Plant Matrices Using LC/MS/MS

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GOOD LABORATORY PRACTICES STATEMENT

This study does not meet the requirements of 40 CFR Part 160.

This study is not required to meet the standards of good laboratory practices since it does not meet the definition of study contained in part 160.3 as there is no test material or experimentation.

Study Director: As this study does not meet the definition of a study as defined in Part 160.3, there is no study director of record.

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Date

Method for Determination of BAS 500 F, BF 500-3 and BAS 510 F Residues in Plant Matrices Using LC/MS/MS

ABSTRACT

Analytical Method No D9908 was developed to determine the residues of BAS 500 F, methyl N-[[[1-(4-chlorophenyl)pyrazol-3-yl]-oxy]-o-tolyl]-n-methoxycarbamate, it's desmethoxy metabolite BF 500-3, and BAS 510 F (2-Chloro-N-(4'-chloro-biphenyl-2-yl)-nicotinamide) in plant matrices. This method measures residues of BAS 500 F, BF 500-3 and BAS 510 F. This method was developed at BASF Corporation, Research Triangle Park, N.C., USA.

This procedure is an abridged version of Method D9908. This original version (BASF Reg. Doc # 2001/5001019) includes two options for extraction and several options for clean up. Version II provides a description of the procedure using the 70/25/5 MeOH/water/2 N HCl extraction, liquid/liquid partition clean up, and an optional silica speedisk® cleanup options. Version II is not a replacement of the original technical procedure but a more concise version including the steps most utilized. This technical procedure is applicable to most plant matrices.

BAS 500 F, its metabolite BF 500-3 and BAS 510 F are extracted with a 70/25/5 methanol/water/2N HCl mixture. An aliquot of the extract is removed and cleaned by liquid/liquid partition. If further cleaning is necessary, a silica speedisk® cleanup may be performed. The final chromatographic analysis of BAS 500 F, its metabolite BF 500-3, and BAS 510 F is performed by LC/MS/MS. The limits of quantitation of the method are 0.02, 0.02 and 0.05 ppm for BAS 500, BF 500-3, and BAS 510 F, respectively.

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1 INTRODUCTION

BAS 500 F and BAS 510 F are fungicides used against several diseases in various crops. Metabolism studies (ref. 1-3) show that the relevant residue consists of the unchanged parent compound for BAS 510 F, and parent and its desmethoxy metabolite BF 500-3 for BAS 500 F. This technical procedure is version II of analytical method D9908 and determines these residues in various plant matrices including (but not limited to): wheat (forage, grain, straw and flour), barley (hay, grain), grape (grapes, raisins, juice), peanut (hay, nutmeat, and oil), dry pea (forage, seeds), lentil seeds, tomato, cucurbits, onion, almond, pecans, carrots, stone fruits, strawberries, raspberry, peppers, mustard greens, and banana (peel and pulp). This method was developed at BASF Corporation, Research Triangle Park, N.C., USA.

2 MATERIALS

Standard substances are stored in a freezer (<-5°C) until use. Information on the characterization of these substances is available from BASF Aktiengesellschaft, Agrocenter, Limburgerhof, Germany.

2.1 List of Abbreviations

ACN	Acetonitrile
LC	HPLC = High Performance Liquid Chromatography
LOQ	Limit of Quantitation
LOD	Limit of Detection
MeOH	Methanol
EtOAC	Ethyl Acetate
DCM	Dichloromethane
MS	Mass Spectrometry
FA	Formic Acid
AF	Ammonium Formate

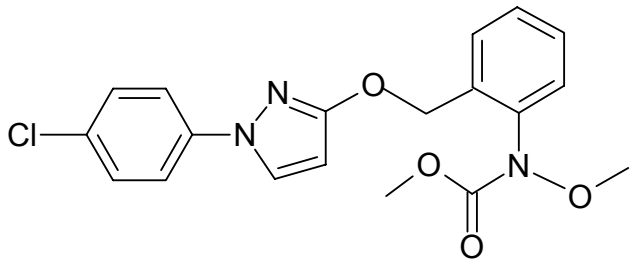
2.2 Reference Substances

Fortification/Reference Compounds

A) BAS 500 F

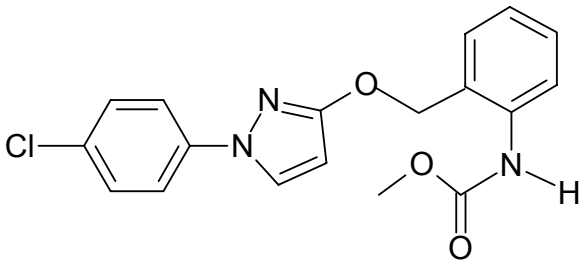
BASF Code Name:	BAS 500 F
BASF Registry Number:	304428
CAS Number:	175013-18-0
Chemical Name:	methyl N-[[[1-(4-chlorophenyl)pyrazol-3-yl]-oxy]-o-tolyl]-n-methoxycarbamate
Molecular Formula:	$C_{19}H_{18}ClN_3O_4$
Molecular Weight:	387.83
Appearance:	White powder
Structural Formula:	

BAS 500 F



B) Metabolite BF 500-3

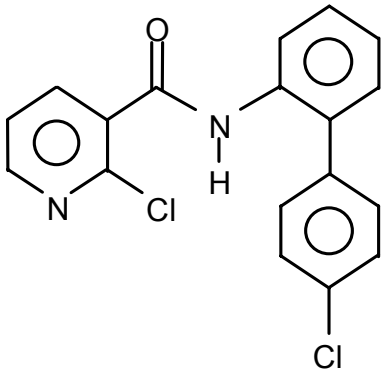
BASF Code Name:	BF 500-3
BASF Registry Number:	340266
Chemical Name:	methyl N-[[[1-(4-chlorophenyl)pyrazol-3-yl]-oxy]-o-tolyl] carbamate
Molecular Formula:	$C_{18}H_{16}ClN_3O_3$
Molecular Weight:	357.8
Appearance	Off-white powder
Structural Formula:	



BF 500-3

C) BAS 510 F

BASF Code Name:	BAS 510 F
BASF Registry Number:	300355
Chemical Name:	2-Chloro-N-(4'-chloro-biphenyl-2-yl)-nicotinamide
Molecular Formula:	$C_{18}H_{12}Cl_2N_2O$
Molecular Weight:	343.21
Appearance	Off-white powder
Structural Formula:	



BAS 510 F

2.3 Equipment -- Suggested Sizes, Suppliers, Manufacturers

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
2.5.2	Spatula	Various	VWR	
3.2	Wide Mouth Bottles	240 mL, Qorpak	VWR	16189-121
Various	Volumetric Flasks	10, 25, 50, 100 mL	VWR	
Various	Culture Tubes	16 X 100 mm	VWR	47729-576
Various	Safe-T-Flex Caps	For 16 mm tubes	VWR	60828-768
Various	PTFE Filter	Acrodisc, 0.45 μm	Pall	4219
Various	Pipettes	0.5 mL, 1 mL, 5 mL, etc.	VWR	
Various	MicroMan pipettes	10 μL – 1000 μL	Rainin	M-25, 50, 250,1000
2.5.2	Analytical Balance	AT261 DeltaRange®	Mettler Toledo	
3.2	Top loading Balance	PG5002 Delta Range	Mettler Toledo	
3.2	Mechanical shaker	HS501digital	Janke & Kunkel	79219
3.2	Polytron	Polytron 3100	Brinkmann	
3.2, 3.3	Multitube Vortexer	VX-2500	VWR	58816-116
3.2, 3.3	Centrivap Concentrator	78100	Labconco	7810002
3.1	SPE Vacuum Manifold	PTFE flow valves	Biotage	121-2016
3.6	LC/MS/MS	API 3000	PE Sciex	

NOTE: Equivalent equipment from other suppliers may be substituted

2.4 Reagents and Chemicals -- Suggested Sources

2.4.1 Chemicals

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Ammonium Formate	MicroSelect >99%	Fluka	09735
Formic Acid	95-97%	Aldrich	10,652-6
Hexane	High Purity	B & J	216-4
Ethyl Acetate	High Purity	B & J	100-4
Dichloromethane	High Purity	B & J	300-4
Acetonitrile	High Purity	B & J	015-4
Methanol	High Purity	B & J	230-4
Cyclohexane	High Purity	B & J	053-4
Sodium Chloride	Reagent	VWR	VW6430-5
Hydrochloric Acid	Reagent	EMD	UN1789
HPLC Grade Water	High Purity	B & J	365-4

NOTE: Equivalent equipment from other suppliers may be substituted

2.4.2 Solvent Mixtures

Solvent Mixture
70/25/5 Methanol/Water/2N HCl (v/v/v)
2 N Hydrochloric acid, 197 g HCl (37%) in 1000 ml H_2O
1 N HCl in Saturated Sodium Chloride
20/80 DCM/Hexane
4/96 Ethyl Acetate/DCM
4 mM Ammonium Formate in 80/20/0.1 Methanol/Water/Formic Acid (v/v/v)

2.5 Standard Solutions

2.5.1 Standard Solution Storage and Stability

Standard solutions are kept refrigerated. Stock solutions (1 mg/mL in ACN or MeOH) should be made fresh every three months. Dilutions of stock solutions should be refrigerated and used no longer than one month.

NOTE: Use amber bottles with Teflon®-lined screw caps as storage containers for standard solutions. Suggested standard concentrations are listed below. A different concentration scheme may be used and additional standards may be prepared as needed.

2.5.2 Standard Solutions of BAS 500 F, BF 500-3, and BAS 510 F for Fortifications

BAS 500 F

Prepare a 1.0 mg/mL BAS 500 F stock solution by weighing an appropriate amount of BAS 500 F into a volumetric flask. Dissolve with ACN or MeOH and dilute to mark. For example, to prepare a 10 mL stock solution, place 10 mg of BAS 500 F into a 10 mL volumetric flask. Dissolve and dilute to mark with solvent.

BF 500-3

Prepare a 1.0 mg/mL BF 500-3 stock solution by weighing an appropriate amount of BF 500-3 into a volumetric flask. Dissolve with ACN or MeOH and dilute to mark. For example, to prepare a 10 mL stock solution, place 10 mg of BF 500-3 into a 10 mL volumetric flask. Dissolve and dilute to mark with solvent.

BAS 510 F

Prepare a 1.0 mg/mL BAS 510 F stock solution by weighing an appropriate amount of BAS 510 F into a volumetric flask. Dissolve with ACN or MeOH and dilute to mark. For example, to prepare a 10 mL stock solution, place 10 mg of BAS 510 F into a 10 mL volumetric flask. Dissolve and dilute to mark with solvent.

Standards of BAS 500 F, BF 500-3, and BAS 510 F for Fortifications

Prepare a 10 μg/mL mixed standard for fortification of BAS 500 F and BF 500-3 by combining 0.5 mL of each stock solution into a 50 mL volumetric flask. Dilute to mark with MeOH.

Prepare a 10 μg/mL solution for BAS 510 F by transferring 0.5 mL of the BAS 510 F stock solution into a 50 mL volumetric flask and dilute to the mark with MeOH.

Prepare serial dilutions of these solutions as needed. Suggested concentrations of standards for fortifications are 10 μg/mL(for a 2 ppm fortification), 100 ng/mL for BAS 500 F and BF 500-3(for 0.02 ppm fortifications), and 250 ng/mL for BAS 510 F (for 0.05 ppm fortifications) in MeOH.

2.5.3 Standard Solutions of BAS 500 F, BF 500-3, and BAS 510 F for LC/MS/MS Analysis

LC/MS/MS Final Solution

The buffer solution is prepared by adding 0.250 grams of Ammonium Formate to 1 liter of 80/20/0.1 MeOH/H2O/Formic Acid.

Mixed Standards for LC/MS/MS Analysis

Prepare a 50 ng/mL mixed standard for LC/MS/MS analysis by combining 0.5 mL of each of the 10 μg/mL solutions of BAS 500 F, BF 500-3, and BAS 510 F fortification solutions into a 100 mL volumetric flask. Dilute to mark with solution containing 4 mM Ammonium Formate in 80/20/0.1 methanol/water/formic acid. Prepare serial dilutions of this combined solution as needed. Suggested concentrations of mixed standards for LC/MS/MS analyses are 0.1 ng/mL, 0.2 ng/mL, 0.5 ng/mL, 1.0 ng/mL, and 2.0 ng/mL in 4 mM Ammonium Formate in 80/20/0.1 methanol/water/formic acid. Other concentration schemes may be used and different or additional standard concentrations may be used if required.

3 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Crop samples are homogenized utilizing a Stephans floor chopper and dry ice. An aliquot is removed from the chopped crop sample and further homogenized in a Retsch Ultra Centrifugal Mill. After homogenization, matrices are stored in a freezer at approximately -15°C until analysis.

3.2 Sample Extraction and Fortification of Plant Matrices

3.2.1 Weighing and Fortification of Non-Oil Matrices

a) Weigh 5 ± 0.1 g of sample material into a 8 oz. wide neck bottle. For the fortification samples, add an appropriate volume of fortification solution to the respective control sample by volumetric or Microman® pipet. For example, an LOQ fortification is prepared by pipetting 1.0 mL of a 250 ng/mL BAS 510 F fortification solution and 1.0 mL of a 100 ng/mL BAS 500 F & BF 500-3 fortification solution onto a control sample. (Other concentration schemes and volumes may be used).

b) Add 100 mL of extraction solvent (70/25/5 Methanol/water/2 N HCl) and agitate on a mechanical shaker @ approximately 200 rpm.

c) Remove approximately 10 mL of the extract (exact volume not necessary) into a 16 X 100 culture tube and centrifuge for 1-5 minutes in a centrivap or swinging bucket centrifuge @ approximately 1500 rpm.

d) Remove a 1.0 mL aliquot of centrifuged extract and Proceed to step 3.3.1.

Optional Additional Filtration Step: For some extremely dirty matrices, for example, cotton gin trash, it may be necessary to add a filtration step after centrifugation. If necessary, filter some of the centrifuged aliquot through a 0.45 μm PTFE syringe filter. Remove aliquot from filtrate.

Note: In order to use a mechanical shaker for extraction, it is essential to have very good sample homogeneity and sample particle size. For example, samples processed through a Retsch Ultra Centrifugal mill equipped with an appropriately sized screen. If samples are not thoroughly processed, after addition of the extraction solvent, samples should be further homogenized using a polytron at 8,000-11,000 rpm for 2-3 minutes. The polytron blades should be rinsed with methanol between samples.

3.2.2 Weighing and Fortification of Oil Matrices

a) Weigh 5 g (±0.2 g) of the oil sample into a 50 mL volumetric flask. For the fortification samples, add an appropriate volume of fortification solution to the respective control sample by volumetric or Microman pipet. For example, an LOQ fortification is prepared by pipetting 1.0 mL of a 250 ng/mL BAS 510 F fortification solution and 1.0 mL of a 100 ng/mL BAS 500 F & BF 500-3 fortification solution onto a control sample. (Other concentration schemes and volumes may be used). Adjust to mark with hexane and mix well.

b) Remove a 1.0 mL aliquot of the extract into a 16 X 100 mm culture tube and add 2.0 mL of ACN. Secure a cap and vortex on a multitube vortexer for one minute @ 2400 rpm.

Note: If an emulsion forms after vortexing, centrifuge for 1-5 minutes in a centrivap or swinging bucket centrifuge @ approximately 1500 rpm.

c) Remove 1.0 mL of the ACN phase into a 16 X 100 mm culture tube and evaporate to dryness in a centrivap @ approximately 60°C and maximum vacuum for 20 minutes (or N-evap @ approximately 45 °C).

d) Reconstitute sample in 1.0 mL 75/25/5 MeOH/H2O/2 N HCl solution and proceed to step 3.3.1.

3.3 Cleanup Procedures Liquid/Liquid Partition

3.3.1 Cyclohexane Liquid/Liquid Partition

Add 1.0 mL of 1 N HCl in saturated NaCl solution and 5.0 mL of cyclohexane to the sample. Secure a cap and vortex on a multitube vortexer for one minute @ 2400 rpm. Allow phases to separate and remove a 1.0 mL from the cyclohexane phase into a 16 X 100 mm culture tube. Evaporate to dryness in a centrivap @ approximately 60°C and maximum vacuum for 20 minutes (or N-evap @ approximately 45 °C). If no further cleaning is required, proceed to step 3.4

Note: The aliquot amount removed from the cyclohexane phase may be modified. Appropriate adjustments to the final volume should be made to insure samples remain within the standard curve (see Section 3.6).

Note: If an emulsion forms after vortexing, centrifuge for 1-5 minutes in a centrivap or swinging bucket centrifuge @ approximately 1500 rpm.

3.3.2 Silica gel Speedisk® Column Cleanup (Optional)

If further cleaning is required, reconstitute sample from 3.3.1 in 5.0 mL 20/80 DCM/Hexane with sonication (15 seconds).

Use an SPE vacuum manifold to perform all the steps for Silica gel Speedisk® column. A solvent flow rate between 8-12 mL/min is usually adequate. Elution profiles for Silica Speedisk may need to be adjusted depending on the lot number of the speedisk. Profiles should be done in the presence of matrix to match the conditions of use as closely as possible and using a sample spiked at 1 ppm or higher for each of the analytes so that any losses in the wash steps or any residue not eluted can be noticed.

a) Condition the Silica Speedisk® column with 2 mL 4%EtOAc/DCM, then 2 mL DCM, and finally 2 mL hexane without allowing the column to go dry.

b) Load sample in 20/80 DCM/hexane to the Silica Speedisk without allowing the column to go dry.

c) Wash the column with 2.0 mL hexane followed by 2.0 mL 20/80 DCM/Hexane. Each of the washes should be passed through the sample tube to provide rinsing.

d) Elute BAS 500 F, BF 500-3 and BAS 510 F with 3.5 mL 4%EtOAc/DCM into an 11 X 13 mm centrifuge tube or other appropriate vessel. Evaporate the samples to complete dryness using the centrivap concentrator @ approximately 60 °C and maximum vacuum for 30 minutes (or N-evap @ approximately 45 °C). Proceed to step 3.4.

3.4 Preparation of the Final Volume for LC/MS/MS Quantitation

A final volume of 1.0 mL is appropriate for control and LOQ fortification samples (0.02 ppm for BAS 500 F and BF 500-3, 0.05 ppm for BAS 510 F). For 2.0 ppm, an appropriate final dilution volume is 40 mL. Use 4 mM Ammonium Formate in 80/20/0.1 MeOH/H_2O/Formic Acid solution for any necessary dilutions.

3.5 LC/MS/MS Instrumentation and Conditions

Instrument:	PE Sciex API 3000 Biomolecular Mass Analyzer		
Inlet [HPLC System]	Perkin Elmer series 200 micro pump + Perkin Elmer 200 Series Auto Sampler		
Column:	Columbus C_{18} 10cm X 2.1 mm, 5μ, 00D-4108-B0		
Injection:	10 μL (or higher)		
Mobile Phase: Gradient	MeOH solution (B) contains: 4 mM Ammonium Formate and 0.1 % Formic Acid Aqueous solution (A) contains: 4 mM Ammonium Formate and 0.1 % Formic Acid		
	Time	A (Aqueous solution)	B (MeOH)
	0.0	60	40
	0.1	60	40
	0.5	10	90
	3.0	10	90
	3.1	60	40
	5.0	60	40
Flow Rate:	300 μL/minute		
Expected Retention Times	**BAS 500 F:** About 2.7 minutes	**BF 500-3:** About 2.8 minutes	**BAS 510 F:** About 2.2 minutes
Ionization Mode:	Positive		
Transitions:	**BAS 500 F:** 388→194 (388→164)	**BF 500-3:** 358→164 (358→132)	**BAS 510 F:** 343→307 (343→140)

NOTE: Suggested LC/MS/MS operating conditions may be modified if necessary.

3.6 Calibration Procedures

Calculation of results is based on peak area (or height) measurements using a calibration curve. The standard curve is obtained by direct injection of the mixed BAS 500 F, BF 500-3 and BAS 510 F standards into LC/MS/MS in the range of 0.1 ng/mL to 2 ng/mL. In a given injection run, the same volume is used for all samples and standards and the correlation coefficient, (r^2), of the standard curve should be 0.98 or higher. Typical standard amounts injected on-column (10 µL) range as follows: 1, 2, 5, 10, 20 pg. Other concentrations may be used as needed.

The calibration curves are obtained by plotting peak area or height (**monitoring transitions 388→194(or 164) for BAS 500 F, transitions 358→164(or 132) for BF 500-3, and transitions 343→307(or 140) for BAS 510 F**) versus the weight or concentration of BAS 500 F or BF 500-3 or BAS 510 F. The linear least squares working curve in the form y = bx + c is used for the construction of the calibration curve.

Establish the stability of the detection response by injecting several concentrations of standards. For each injection set, the set should begin and end with standard injections, and each standard level should be injected at least in duplicate.

3.7 Limit of Quantitation and Limit of Detection

The limit of quantitation is defined as the lowest fortification level successfully tested. For all matrices, the limit of quantitation is 0.02 ppm for BAS 500 F and BF 500-3 and 0.05 ppm for BAS 510 F. The limit of detection is 0.04 pg/µL (concentration on the LC column), which is 40% of the lowest standard injected into the LC/MS/MS instrument.

4 CALCULATION OF RESULTS

4.1 Principle

Calculation of results is based on peak area (or height) measurements. The residues of BAS 500 F, BF 500-3, and BAS 510 F are calculated from the calibration curve and the equations shown in Section 4.2.

4.2 Calculation of Residues

The residues of **BAS 500 F, BF 500-3, or BAS 510 F** in mg/kg (ppm) are calculated with the following formula and expressed as parent equivalents.

$$\text{Residue (ppm)} = \frac{V_E \times W_A \times MWCF}{G \times A_F \times V_I \times 1000} \quad \text{or} \quad \frac{V_E \times C_A \times MWCF}{G \times A_F \times 1000}$$

V_E = Final Volume after dilutions (mL)
W_A = Amount of analyte from calibration curve (pg)
C_A = Concentration of Analyte from calibration curve (ng/mL)
G = Sample weight extracted (g)
A_F = Aliquot factor
V_I = Injection volume (µL)
1000 = Factor remaining after all unit conversions

MWCF(Molecular Weight Conversion Factor) : = 1 for BAS 500 F and BAS 510 F
= 1.08 to convert BF 500-3 to BAS 500 F

4.3 Calculation of Recoveries

The recoveries of **BAS 500 F, BF 500-3, and BAS 510 F fortification** samples are calculated with the following formula.

$$\text{Residue (ppm)} = \frac{V_E \times W_A \times MWCF}{G \times A_F \times V_I \times 1000} \quad \text{or} \quad \frac{V_E \times C_A \times MWCF}{G \times A_F \times 1000}$$

V_E = Final Volume after dilutions (mL)
W_A = Amount of analyte from calibration curve (pg)
C_A = Concentration of Analyte from calibration curve (ng/mL)
G = Sample weight extracted (g)
A_F = Aliquot factor
V_I = Injection volume (µL)
1000 = Factor remaining after all unit conversions

Recovery % is calculated as follow:

$$\text{Recovery \%} = \frac{\text{Residue in fortified sample (ppm) - Residue in control (ppm)}}{\text{Amount analyte fortified (ppm)}} \times 100$$

5 TIME REQUIREMENT FOR ANALYSIS

The time required for a set of 22 control and treated samples and 2 recoveries is approximately 8 person-hours not including LC/MS/MS analysis. Calculations and data entry following LC/MS/MS analysis take less than one hour provided high residue samples do not require excessive dilutions. These time estimates are appropriate provided that no special problems arise, such as matrix interference.

6 CONFIRMATORY TECHNIQUES

The LC/MS/MS final determination for BAS 510 F, BAS 500 F, BF 500-3 is a highly selective detection technique. Two separate transitions are monitored, one which can be used for quantitative evaluation, the other transition can be used for confirmation of residue findings. Therefore, no additional confirmatory technique is required

7 POTENTIAL PROBLEMS

In the event of poor recoveries, a control spike (control sample reconstituted in 1.0 mL of an injection standard) should be run to measure instrument performance. If enhancement or suppression is observed, use the optional clean up step.

8 SAFETY AND HEALTH CONSIDERATIONS

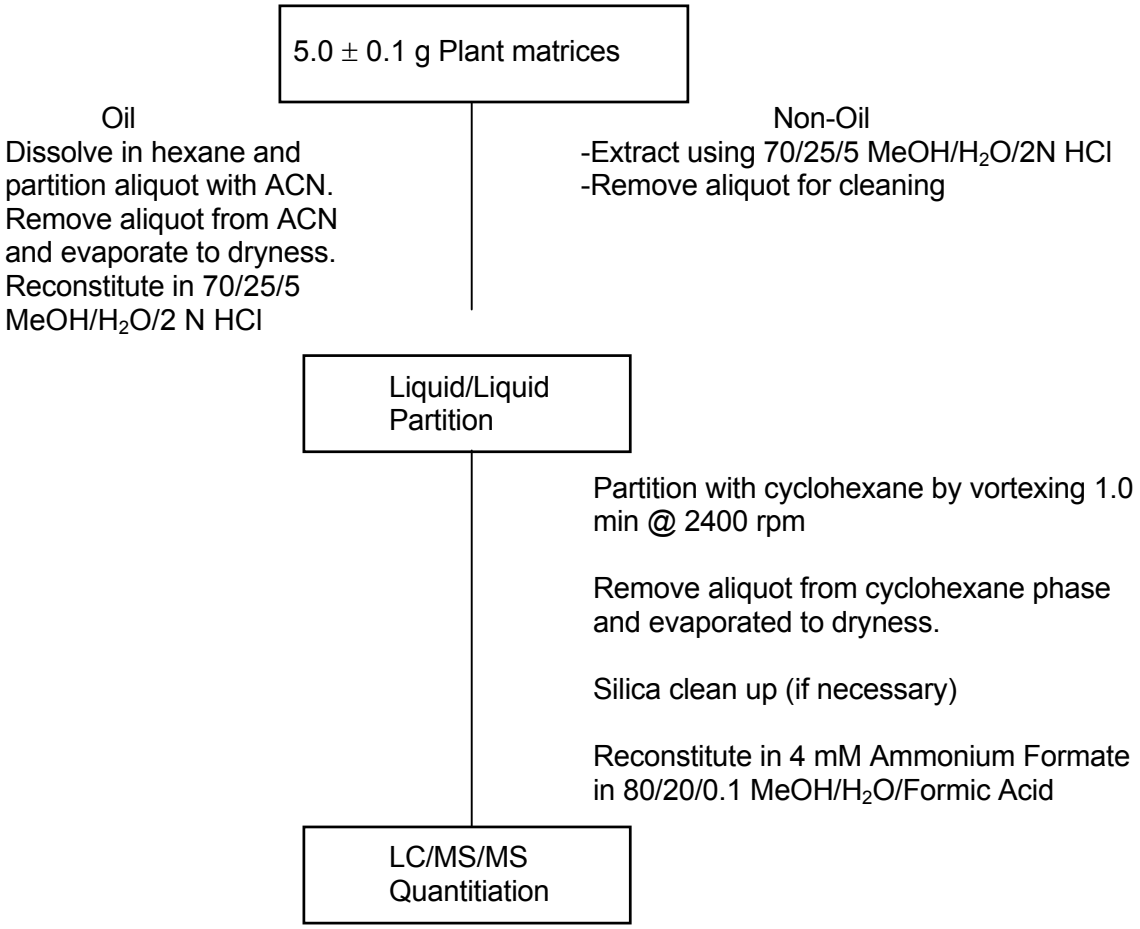
All procedures involving organic solvents should be performed under a well-ventilated hood. Personal protective equipment (gloves, lab coats) should be worn while performing this method. Heed all label statements and precautions.

9 REFERENCES

1) Abdel-Baky S., Jones J. “Method for Determination of BAS 500 F, BF 500-3 and BAS 510 F Residues in Plant Matrices using LC/MS/MS”, BASF Reg. Doc No. 2001/5001019.

10 FLOW CHART FOR THE ANALYTICAL METHOD

Flow Chart for the Analytical Procedure For BAS 500 F, BF 500-3 and BAS 510 F In Plant Matrices



APPENDIX

A) Calculation of Residues [Example]

Spike sample at 0.02 ppm:

BAS 500 F and BF 500-3 (pg) interpolated from standard curve:

Standard curve: (pg) BAS 500 F, BF 500-3 = $\frac{\text{Response - Intercept}}{\text{Slope}}$

	BAS 500 F	BF 500-3
Response:	7650	9901
Slope:	5190	6010
Intercept:	79	-316

$$\text{(pg) BAS 500 F} = \frac{7650 - 79}{5190} = 1.46 \text{ pg}$$

$$\text{(pg) BF 500-3} = \frac{9901 + 316}{6010} = 1.70 \text{ pg}$$

Following values were used in this analysis:

Sample Weight = 5.0 g (G)
Final Volume = 1 mL (for 0.02 ppm) (V_E)
Injection Volume = 10 µL (V_I)
Aliquot = 0.002 (A_F)
MWCF = 1.0 for BAS 500 F and BAS 510 F,
= 1.08 to convert BF 500-3 to BAS 500 F

Amount of Analyte Found (from above):
BAS 500 F= 1.46 pg, BF 500-3 = 1.70 pg (W_A)

$$\text{BAS 500 F Residue (ppm)} = \frac{1 \text{ mL} \times 1.46 \text{ pg} \times 1.0}{5.0 \text{ g} \times 0.002 \times 10 \text{ µL} \times 1000} = 0.0146$$

$$\text{BF 500-3 Residue (ppm)} = \frac{1 \text{ mL} \times 1.70 \text{ pg} \times 1.0}{5.0 \text{ g} \times 0.002 \times 10 \text{ µL} \times 1000} = 0.0170$$

NOTE: For Treated Samples, BF 500-3 residue is multiplied by 1.08 to convert to BAS 500 F equivalents. This correction is not necessary for recovery samples.

B) Calculation of Recoveries [Example]

Spike sample at 0.02 ppm:

$$\text{Recovery \% (BAS 500 F)} = \frac{0.0146 \text{ ppm} - 0.0 \text{ ppm in control}}{0.02 \text{ ppm}} \times 100 = 73\%$$

$$\text{Recovery \% (BF 500-3)} = \frac{0.0170 \text{ ppm} - 0.0 \text{ ppm in control}}{0.02 \text{ ppm}} \times 100 = 85\%$$