UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: July 2, 2003

Reviewers:

William T. Drew, Chemist — Date: 8/20/03
Reviewer
RAB2/HED (7509C)

Henri P. Bietlot, Chemist — Date: July 16/03
Peer reviewer
FREAS, HED, PMRA

Richard A. Loranger — Date: 8/15/03
Branch Senior Scientist
RAB2/HED (7509C)

Ariff Ally — Date: July 25/03
Section Head
FREAS, HED, PMRA

DP Barcode: D278386

Petition: 1F06313

Citation: 45405027 Jones, J. (2001) Method for Determination of BAS 500 F, BF 500-3, and BAS 510 F Residues in Plant Matrices Using LC/MS/MS: Final Report: Lab Project Number: 64692: 2001/5001020: D9908. Unpublished study prepared by BASF Agro Research. 79 pages.

Sponsor: BASF Corporation

Background:

The information contained herein was compiled by Dynamac Corporation (20440 Century Boulevard, Suite 100, Germantown MD 20874), contractor, under the supervision of RAB2/HED. This DER has undergone secondary review by RAB2, and reflects current HED and Office of Pesticide Programs (OPP) policies. This DER has also been peer-reviewed by PMRA/Canada.

Executive Summary

BASF Corporation has proposed **LC/MS/MS method D9908** for the purposes of data collection of residues of BAS 510 F in plant commodities. Briefly, samples are extracted with ACN,

methanol:water, methanol:water:HCl, or hexane (depending on sample matrix), then cleaned up by liquid/liquid partitioning, C18 solid phase extraction and/or silica gel solid phase extraction, after which residues are determined by LC/MS/MS. MS/MS detection using the positive ionization mode monitors ion transitions from *m/z* 343 to 307 for BAS 510 F, *m/z* 388 to 194 for BAS 500 F (pyraclostrobin), and *m/z* 358 to 164 for BF 500-3 (a metabolite of pyraclostrobin). Quantitation is obtained using an external calibration curve of BAS 510 F standards. The limit of quantitation (LOQ) is 0.050 ppm for residues of BAS 510 F in/on plant matrices. The LOD was quoted as 5 pg/µl.

Method validation was conducted for this LC/MS/MS method in three representative plant matrices, almond nutmeat, onion, and plum. Method validation recoveries for residues of BAS 510 F ranged 73-85% in almond nutmeat, 67-113% in onion, and 93-97% in plum fortified with BAS 510 F at 0.050 and 3.00 ppm. Although some of the recoveries at the method LOQ were outside the recommended 70-120% range, overall recoveries were acceptable.

A study to demonstrate the stability of standard solutions of BAS 510 F and various of its metabolites in ACN has been submitted separately (see DER of MRID 45405104). The recoveries of BAS 510 F, M510F01, M510F49, M510F51, and M510F53 following 62 days of storage, either refrigerated in the dark or at room temperature with daylight exposure, indicated no loss of concentration during storage. Based on these data, the petitioner recommended that standard solutions be stored no longer than 60 days. **As a condition of registration,** the petitioner should revise this LC/MS/MS method to recommend a 60-day maximum storage interval for standard solutions of reference standards, and submit a copy of the revised method to the Agency.

Provided the method is revised to impose a 60-day limitation on the storage of standard solutions of reference standards, and provided concurrent method validations are conducted in conjunction with the field samples analyzed by this LC/MS/MS method, it will be considered acceptable for data collection purposes.

A separate GC/MS method (Method D0008) is proposed as the enforcement method for residues of BAS 510 F in/on plant matrices (refer to the DER for MRIDs 45405028 and 45405101).

It is noted that Method D9908 also determines residues of pyraclostrobin and its metabolite BF 500-3 in plant commodities, and the current submission includes method validation data for pyraclostrobin and its metabolite. These data are not reviewed herein as they are not applicable to guideline requirements for BAS 510 F.

GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided.

1. Materials and Methods

1.1. Test Substances

Table 1.1.1. List of Analytes Tested with the LC/MS/MS Method.	
Common Name:	Nicobifen (ISO, proposed)
IUPAC Name:	2-Chloro-N-(4'-chlorobiphenyl-2-yl)-nicotinamide
CAS Name:	3-Pyridinecarboxamide, 2-chloro-N-(4'chloro[1,1'-biphenyl]-2-yl)-
CAS Number:	188425-85-6
Company Name:	BAS 510 F
Other Synonyms:	BASF Registry No. 300355

Table 1.1.2. Matrices Tested with the LC/MS/MS Method.	
Matrix	Matrix Form
Almond	Untreated samples obtained from a field trial (BASF Study 94047). Samples were homogenized with dry ice and frozen until extraction and analysis.
Onion	Untreated samples obtained from field trials (BASF Studies 78035 and 64250). Samples were homogenized with dry ice and frozen until extraction and analysis.
Plum	Untreated samples obtained from a field trial (BASF Study 96070). Samples were homogenized with dry ice and frozen until extraction and analysis.

Table 1.1.3. Fortification Levels Tested with the LC/MS/MS Method.	
Matrices	BAS 510 F (ppm)
Almond, onion, and plum	0.050 (LOQ)
	3.00

1.2. Methods

A description of Method D9908 follows. For method validation, homogenized samples of almond, onion, and plum were fortified with a solution of BAS 510 F in acetonitrile (ACN). Samples were fortified prior to method extraction.

For matrices other than oil, Method D9908 allows two types of extractions: extraction using Polytron homogenizer or extraction using accelerated solvent extraction (ASE). For extraction using a homogenizer, samples are homogenized with methanol:water:2 N HCl (70:25:5, v:v:v). For ASE extraction, an ASE cell is packed with sand, the test plant matrix, and then additional sand. Residues are extracted with ACN for oily matrices (nutmeats) or methanol:water (75:25, v:v) for non-oily matrices; extraction is conducted at 50° C and 2000 psi. In the case of ACN extraction, an aliquot of the extract is washed twice with hexane, evaporated to dryness, and

redissolved in methanol, 0.1% formic acid, and water. For methanol:water extracts, an aliquot is diluted with water.

Oil samples are extracted by mixing with hexane. An aliquot of the hexane mixture is partitioned with ACN and an aliquot of the ACN phase is diluted with water and concentrated.

The extracts are then subjected to cleanup using liquid/liquid partitioning (only for oil samples and samples extracted using a homogenizer) or solid phase extraction (SPE). For liquid/liquid partitioning, the extract is mixed with saturated sodium chloride solution, concentrated HCl, and cyclohexane. An aliquot of the cyclohexane fraction is either evaporated to dryness (if no interference is expected) and dissolved in LC/MS/MS mobile phase for analysis or mixed with dichloromethane:hexane (20:80, v:v) for further cleanup by C18 and/or silica gel SPE. For SPE cleanup, the extract is applied to a C18 SPE column; residues are eluted with dichloromethane and evaporated to dryness. Residues are either dissolved in LC/MS/MS mobile phase for analysis or dissolved in dichloromethane:hexane (20:80, v:v) and cleaned up further using silica gel SPE. Residues are eluted from the silica gel SPE with 4% ethyl acetate in dichloromethane. The eluate is evaporated to dryness and dissolved in LC/MS/MS mobile phase for analysis.

The LC system utilizes an Intersil Phenyl 5μm column and an isocratic mobile phase of methanol:4 mM ammonium formate:0.1% formic acid (80:19.9:0.1, v:v:v). MS/MS detection using the positive ionization mode monitors ion transitions from *m/z* 343 to 307 for BAS 510 F, *m/z* 388 to 194 for BAS 500 F, and *m/z* 358 to 164 for BF 500-3. Quantitation is obtained using an external calibration curve of BAS 510 F standards.

For the samples used for method validation, almond nutmeats were extracted by ASE using ACN and subjected to cleanup using both C18 and silica gel SPE, plum samples were extracted using a homogenizer and subjected to cleanup using C18 SPE only, and onion samples were extracted using a homogenizer and subjected to cleanup by liquid/liquid partitioning and silica gel SPE.

2. Results

2.1. Stability of Reference Materials

The reference substance was stored frozen (<-5° C) until used in the validation study. Stock solutions of the BAS 510 F standard in ACN or methanol were stored refrigerated except when in use. The method recommends that stock solutions of the standard be made fresh every three months, and that dilutions of the stock solutions be stored refrigerated for no longer than one month. Fortification standards were prepared in ACN from stock solutions in ACN, and calibration standards were prepared with 4 mM ammonium formate:0.1% formic acid buffer (99.9:0.1, v:v) from stock solutions in methanol.

A study to demonstrate the stability of standard solutions of BAS 510 F and various of its metabolites in ACN has been submitted separately (see DER of MRID 45405104). The

recoveries of BAS 510 F, M510F01, M510F49, M510F51, and M510F53 following 62 days of storage, either refrigerated in the dark or at room temperature with daylight exposure, indicated no loss of concentration during storage. Based on these data, the petitioner recommended that standard solutions be stored no longer than 60 days. **As a condition of registration,** the petitioner should revise this LC/MS/MS method to recommend a 60-day maximum storage interval for standard solutions of reference standards, and submit a copy of the revised method to the Agency.

2.2. Method Characteristics

2.2.1. Chromatography

The representative LC/MS/MS total ion chromatograms for almond, onion, and plum samples indicate that the peak shape was generally good for BAS 510 F. Small peaks were observed in the control samples of almond nutmeats and onions; however, these peaks were present at levels below the method LOQ (it could not be determined whether the peaks were below the stated limit of detection).

2.2.2. Linearity

Adequate method linearity was observed. The coefficient of determination (r^2) from a representative calibration standard curve was 0.998 for BAS 510 F standard concentrations ranging 0.1-10.0 pg/µL.

2.2.3. Specificity

An interference study is not required as the method employs an MS/MS detector (m/z 343 to 307). Although small interferences were observed in some chromatograms, these interferences were below the LOQ.

2.2.4. Method Limits

The limit of detection (LOD) was 0.50 pg/µL (concentration on the LC column), which is the lowest standard injected onto the LC/MS/MS system. The stated limit of quantitation (LOQ) was 0.050 ppm for the residues of BAS 510 F in/on all plant matrices.

2.2.5. Analyte Recoveries

Table 2.2.5.1. Recovery of BAS 510 F from Plant Matrices Using the LC/MS/MS Data Collection Method.

Matrix	Fortification Level, ppm	Recoveries, %	Mean ± SD, % (n)
Almond nutmeat	0.050 (LOQ)	73[1], 73, 84[1], 84	81 ± 5 (4)
	3.00	61[1], 67[1], 82, 85	
Onion	0.050 (LOQ)	67, 67, 98, 113	86 ± 18 (6)
	3.00	81, 88	
Plum	0.050 (LOQ)	97, 97[1]	95 ± 2 (4)
	3.00	93, 94	

[1] The petitioner noted that generally low recoveries were observed with this sample set due to problems with the ASE cell, therefore, the set was re-analyzed. These initial recoveries were not included in the mean and standard deviation calculations.

2.2.6. Independent Laboratory Validation

Independent laboratory validation (ILV) is not required for LC/MS/MS method D9908 because it is used only for data collection. A separate **GC/MS** method, **Method D0008**, has been proposed as the enforcement method; method validation and ILV data have been submitted in support of the enforcement method (refer to the DER for MRIDs 45405028 and 45405101).

3. Discussion

3.1. Recovery and Repeatability

Method validation was conducted for the LC/MS/MS method for analysis of residues of BAS 510 F in three representative plant matrices, almond nutmeat, onion, and plum. Method validation recoveries for residues of BAS 510 F ranged 73-85% in almond nutmeat, 67-113% in onion, and 93-97% in plum fortified with BAS 510 F at 0.050 and 3.00 ppm.

Although some of the recoveries at the method LOQ were outside the recommended 70-120% range, overall recoveries were acceptable. **As long as concurrent method validations are conducted in conjunction with the field samples** analyzed using the LC/MS/MS method D9908, the method will be considered acceptable for data collection purposes.

3.2. Method Efficiency

Radiovalidation was not conducted for this method. Because the plant metabolism studies (see DERs for MRIDs 45405021-45405023) indicated that residues were readily extractable with methanol, radiovalidation of the proposed enforcement method will not be required.

4. Deficiencies

As a condition of registration, this analytical method (LC/MS/MS method D9908) should be revised to recommend a 60-day maximum storage interval for standard solutions of reference standards. A copy of the revised method should be submitted to the Agency.

5. References

None.