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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: July 2, 2003

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DP Barcode: D278386

Petition: 1F06313

Citation: 45405105 Fabian, E. (2001) The Validation of BASF Method 476/0: The Determination of BAS F Residues (as M510F53) in Liver and Milk by Microwave Treatment: Final Report: Lab Project Number: 96997: 476/0: 2000/1017224. Unpublished study prepared by BASF Aktiengesellschaft. 42 pages.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 submitted a data collection method, **GC/MS method 476/0**, for the determination of bound residues of BAS 510 F in milk and liver. Method 476/0 was developed

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to determine nonextractable residues of BAS 510 F in liver and milk. The method is a common moiety method based on the quantification of the metabolite M510F53. During the goat and rat metabolism studies (MRIDs 45405125 and 45404918, respectively), it was observed that residues of BAS 510 F were bound to liver proteins through substitution of chlorine in the pyridine ring with thiol groups of proteins. Microwave hydrolysis experiments revealed that this conjugation modified the reactivity of the whole molecule, allowing the amide bond to be cleaved during microwave treatment. If the microwave hydrolysis was conducted using acetic acid, metabolite M510F53 could be generated. The hydrolysis of the amide bond was not observed during microwave treatment of the parent or its metabolite M510F01. Based on these results, M510F53 is used as a marker analyte for bound residues of BAS 510 F in liver. The microwave hydrolysis techniques were also applied to milk samples in the goat metabolism study, theoretically releasing conjugated residues of BAS 510 F.

Using Method 476/0, residues in liver and milk are mixed with ACN and concentrated acetic acid, treated by microwave extraction at 170 C for 0.5 hours, and concentrated to a crude acetic acid solution. For method validation purposes, fortifications of M510F53 were made at this point. Saturated sodium chloride was added, the pH adjusted to ~12, and residues extracted (2x) with iso-octane. The organic extract was cleaned-up by silica gel and/or sequential C18 solid phase extraction (SPE). The residues were evaporated to dryness and redissolved in ACN for GC/MS analysis. The MS detector uses selected ion monitoring (SIM); ion *m/z* 167 was detected for M510F53. Quantitation is obtained using an external calibration curve of M510F53 standards.
The validated limit of quantitation (LOQ) for the residues of M510F53 was 0.010 ppm in milk and 0.050 ppm in cow liver.

Method validation was conducted for the GC/MS microwave method 476/0 for analysis of residues of M510F53 in milk and cow liver. Method validation recoveries ranged 91-105% in milk fortified with M510F53 at 0.010 and 0.10 ppm, and 88-102% in cow liver fortified with M510F53 at 0.050 and 0.50 ppm. Overall recoveries were acceptable for residues of M510F53 in milk and liver. However, it was noted that analyte fortifications were made after the microwave hydrolysis step. Although not explicitly stated, it was presumed that the petitioner did not have any reference standards available to demonstrate the ability of the method to hydrolyze conjugated residues.

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 GC/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.

Radiovalidation was not conducted for this method. Because the method involves a hydrolysis step which has not been validated with fortification standards, radiovalidation data should be submitted as a **condition of registration**.

Contingent upon the submission of radiovalidation data demonstrating the efficiency of the microwave hydrolysis step, **and provided** the method is revised to impose a 60-day limitation on the storage of standard solutions of reference standards, this method is considered adequate for the purposes of collecting data on bound residues of BAS 510 F in liver and milk.

GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. The petitioner stated that the study was conducted in accordance with the GLP regulations established in Germany (Appendix 1 to §19a Section 1, Chemikaliengesetz of 25-July-1994; Official Bulletin/Federal Republic of Germany I 1994, page 1703) instead of U.S. EPA GLP regulations or PMRA's GLP reguierments.

1. Materials and Methods

1.1. Test Substances

Table 1.1.1. List of Analytes Tested with the GC/MS Microwave Method.	
Common Name:	None assigned (Marker analyte for bound BAS 510 F residues)
IUPAC Name:	N-(4'-chlorobiphenyl-2-yl)acetamide
CAS Name:	Not available
CAS Number:	Not available
Company Name:	M510F53
Other Synonyms:	BASF Registry No. 4035210

Table 1.1.2. Matrices Tested with the GC/MS Microwave Method.	
Matrix	Matrix Form
Milk	Purchased commercially.
Cow liver	Purchased commercially and homogenized.

Table 1.1.3. Fortification Levels Tested with the GC/MS Microwave Method.	
Matrices	M510F53 (ppm)
Milk hydrolysate	0.010 (LOQ)
	0.10
Cow liver hydrolysate	0.050 (LOQ)
	0.50

1.2. Methods

Method 476/0 was developed to determine nonextractable residues of BAS 510 F in liver and milk. The method is a common moiety method based on the quantification of the metabolite M510F53. During the goat and rat metabolism studies (MRIDs 45405125 and 45404918, respectively), it was observed that residues of BAS 510 F were bound to liver proteins through substitution of chlorine in the pyridine ring with thiol groups of proteins. Microwave hydrolysis experiments revealed that this conjugation modified the reactivity of the whole molecule, allowing the amide bond to be cleaved during microwave treatment. If the microwave hydrolysis was conducted using acetic acid, metabolite M510F53 could be generated. The hydrolysis of the amide bond was not observed during microwave treatment of the parent or its metabolite M510F01. Therefore, M510F53 is used as a marker analyte for bound residues of BAS 510 F in liver. The microwave hydrolysis techniques were also applied to milk samples in the goat metabolism study, theoretically releasing conjugated residues of BAS 510 F.

A description of method 476/0 follows. For method validation, samples of milk and cow liver hydrolysates were fortified with M510F53 in acetonitrile (ACN) after the microwave hydrolysis step.

Briefly, milk is mixed with ACN and an aliquot is taken for analysis. Homogenized liver is combined with ACN. The milk ACN aliquot and liver ACN homogenate are placed into microwave containers. Concentrated acetic acid is added and the solutions subjected to microwave treatment: the temperature is elevated to 170 C over a period of 5 minutes and maintained at 170 C for 0.5 hours. The samples are cooled, left for two days at ambient conditions, and then concentrated to a crude acetic acid solution. Fortifications with M510F53 are made at this point. Saturated sodium chloride is added, the pH adjusted to ~12 with 10 M potassium hydroxide, and residues extracted (2x) with iso-octane. The organic extract is filtered through sodium sulfate, evaporated to dryness under reduced pressure, and residues redissolved in iso-octane for cleanup through a silica gel solid phase extraction (SPE) cartridge. Residues are eluted with iso-octane:acetone (9:1 for liver or 8:2 for milk, v:v) under vacuum and evaporated to dryness. Liver residues are redissolved in methanol:water (3:7, v:v) and subjected to additional cleanup through a C18 SPE cartridge; milk residues do not need C18 SPE cleanup. Residues are eluted with methanol:water (6:4, v:v) under vacuum and evaporated to dryness. The residues are redissolved in ACN for GC/MS analysis. The MS detector uses selected ion monitoring (SIM);

ion *m/z* 167 was detected for M510F53. Quantitation is obtained using an external calibration curve of M510F53 standards.

2. Results

2.1. Stability of Reference Materials

Fortification solutions of the M510F53 standard were prepared in ACN and calibration solutions of the M510F53 standard were prepared in methanol. The storage conditions of the fortification and standard solutions were not addressed.

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 GC/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 GC/MS spectra for milk and cow liver samples indicate that the peak shape was generally good for M510F53. No interferences were observed in the control samples.

2.2.2. Linearity

The method linearity was good. The coefficient of determination (r^2) was not reported but a representative calibration standard curve for M510F53 standard concentrations ranging 0.02-1.0 ppm was included.

2.2.3. Specificity

An interference study is not required as the method employs a MS detector. No interferences were observed in the unfortified samples.

2.2.4. Method Limits

The limit of detection (LOD) was not determined. The validated limit of quantitation (LOQ) for the residues of M510F53 was 0.010 ppm in milk and 0.050 ppm in cow liver.

2.2.5. Analyte Recoveries

Table 2.2.5.1. Recovery of M510F53 From Milk and Liver Using the GC/MS Microwave Method 476/0.

Matrix	Fortification Level (ppm)	Recoveries (%)	
		M510F53	Mean ± SD
Milk	0.010	91, 92, 94, 96, 102	98 ± 5
	0.10	94, 98, 100, 103, 105	
Liver	0.050	88, 89, 92, 92, 94	94 ± 5
	0.5	94, 95, 100, 100, 102	

2.2.6. Independent Laboratory Validation

ILV is not required for GC/MS microwave method 476/0 because it is used only for data collection. A separate **GC/ECD** method is 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 45405102 and 45405103).

3. Discussion

3.1. Recovery and Repeatability

Method validation was conducted for the GC/MS microwave method 476/0 for analysis of residues of M510F53 (as a marker analyte for bound residues) in milk and cow liver. Method validation recoveries ranged 91-105% in milk fortified with M510F53 at 0.010 and 0.10 ppm, and 88-102% in cow liver fortified with M510F53 at 0.050 and 0.50 ppm.

It was noted that analyte fortifications were made after the microwave hydrolysis step. Although not explicitly stated, it was presumed that the petitioner did not have any reference standards available to demonstrate the ability of the method to hydrolyze conjugated residues.

3.2. Method Efficiency

Radiovalidation was not conducted for this method. Because the method involves a hydrolysis step which has not been validated with fortification standards, radiovalidation data should be submitted as a condition of registration.

4. Deficiencies

As a condition of registration, this GC/MS analytical method 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.

As a condition of registration, radiovalidation data to demonstrate the efficiency of the method to determine bound/conjugated residues should be submitted.

5. References

None.

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