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Final Study Report:

**Independent Method Validation of BASF Analytical Method D0307 entitled  
"Analytical Method for the LC/MS/MS Determination of Imazapic (BAS 715 H) and  
Metabolite CL 354825 in Soil"**

Data Requirement:

EPA Pesticide Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry  
Methods (Subdivision N 164-1. Environmental Chemistry Method)

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## I. INTRODUCTION

### A. Purpose of Study

This study was conducted to fulfill EPA Pesticide Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods (Subdivision N 164-1, Environmental Chemistry Method). This study was conducted to determine if Method D0307 can be performed successfully by an individual unfamiliar with the method or its development.

## II. MATERIALS / METHODS

### A. Test and Reference Substance:

BAS Code:	<b>BAS 715 H</b>
Common Name :	Imazapic
Chemical Name	Nicotinic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methyl-
Lot Number	AC10606-119
Purity	99.3%
Exp Date	February 21, 2006

BAS Code	<b>CL 354825</b>
Common Name :	5-carboxy-3-hydroxy pyridine imidazolinone
Chemical Name	Nicotinic acid, 5-hydroxy-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin -2-yl)
Lot Number	AC9918-101
Purity	90%
Exp Date	October 17, 2006

The test/reference substances were maintained frozen (<-5° C to <-15° C) until their use in this study. Characterization and purity were determined prior to the substances being used in this study. Details of these determinations are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

The test/reference substances were used in the study to generate data for both instrument and method performance. Quantitation of all samples was achieved using calibration curves calculated by linear regression of instrument responses for the reference substance. The performance of the instrument was evaluated during each injection set. The correlation coefficient of the calibration curve was required to be at or above 0.99.

Test substance solutions for fortifications were refrigerated during their use in this study. BAS 715 H and CL 354825 stock solutions were made fresh in methanol. Mixed fortification solutions of BAS 715 H and CL 354825 were made fresh from the mixed 10.0 µg/mL standard solution by diluting with water.

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Reference substance solutions for instrument calibration were refrigerated during their use in this study. Mixed calibration solutions of BAS 715 H and CL 354825 were made fresh from the mixed 0.1 µg/mL fortification solution by diluting in water.

#### **B. Test System**

Untreated soil, obtained from a method validation study (study 121235, Reference 1) was the test system used for this study. Method D0307 was developed for the analysis of residues of BAS 715 H and its metabolite CL 354825 in soil.

#### **C. Identification of Samples**

Untreated soil was obtained from BASF Agro Research Study Number 121235. In the earlier study, the control soil was homogenized and then stored frozen in a plastic bag. For this study, the soil was assigned a unique residue sample number (RSN): 2003026-1

Each analysis set was uniquely identified with a Master Sheet Number which consisted of the study number plus a unique number (e.g., 92327-01).

#### **D. Method of Analysis**

Fortified soil samples were analyzed using BASF Analytical Method D0307. Minor changes were made to the instrumental parameters indicated in the method. These are listed below. In all other aspects, the method was followed as written. A brief description of the methodology follows.

Residues of BAS 715 H and CL 354825 are extracted from soil using 0.5 N NaOH. An aliquot of the extract is treated with formic acid to precipitate the humic material. After cleanup of the acidified extract using C18 solid phase extraction (SPE) and, if needed SCX SPE, the sample is analyzed by LC/MS/MS with quantitation of both analytes in the positive mode. The results are calculated as BAS 715 H and CL 354825 by direct comparison of the sample peak responses to those of external standards. The limit of quantitation (LOQ) of the method for each analyte is 0.001 ppm.

The instrument conditions were modified slightly for both trials. Due to instrument availability, a Sciex API 4000 LC/MS/MS instrument was used in the second trial instead of the API 3000, suggested in the method. For both trials, the collection window was extended by 30 seconds to optimize signal intensity. In the second injection of the second trial, an extra 1.5 minutes was added to the instrument equilibration time after the gradient to reduce shifting retention times.

Equations used to calculate residues and recoveries are included in the method. Example calculations are also included in the method.

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#### **E. Validation of Method**

To validate the method independently, two controls and five fortifications at the limit of quantitation (0.001 ppm) and five fortifications at ten times the limit of quantitation (0.01 ppm) were analyzed for each of the compounds. The method is validated, as per EPA Pesticide Residue Guidelines, when the average recovery at each fortification level is between 70 and 120%, the coefficients of variation are less than 20%, the control values are less than 20% of the LOQ and the correlation coefficient of the standards is at least 0.99.



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## V. DISCUSSION / CONCLUSIONS

The method validation method D0307 was run successfully in two method trials. The first trial failed due to laboratory contamination of samples. The results from the second trial were acceptable.

The following suggestions for the Method D0307 are recommended as a result of this independent validation:

**Section 2.5.2.3 - (second paragraph)** state the concentration of the source BAS 715 H and CL 354825 solution in the example:

"For example, to prepare 100 mL of the mixed solution, pipette 1 mL each of the BAS 715 H and CL354825 1mg/mL stock solutions into a 100-mL volumetric flask. Add methanol, mix well and dilute to mark with methanol."

**Section 3.5.1 - (first paragraph, last sentence)** two typographical errors:

"...and leave a shallow layer of 0.10 H HCl over the to frit of the cartridge." should read

"...and leave a shallow layer of 0.10 N HCl over the frit of the cartridge."

**Section 3.6** – It should be noted that if shifting retention times become troublesome, the instrument equilibration time after the gradient may be extended by 30 seconds. It should also be noted that the instrument collection window should be checked and adjusted, if necessary, when first setting up this method on the instrument, to optimize signal intensity.

Caution should be included about avoiding contamination by using care while handling concentrated standards and by thorough cleaning of the SPE apparatus.

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There were no protocol changes to this study.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at BASF inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples and test and reference substances were maintained in secured (i.e. pad-locked) storage with limited access. Freezer temperatures were continuously monitored by electronic means. The final report, protocol, and raw data pertaining to this study are maintained at the BASF Agro Research, 26 Davis Drive, Research Triangle Park, North Carolina.

Figure 1 Flow Diagram of Method D0307

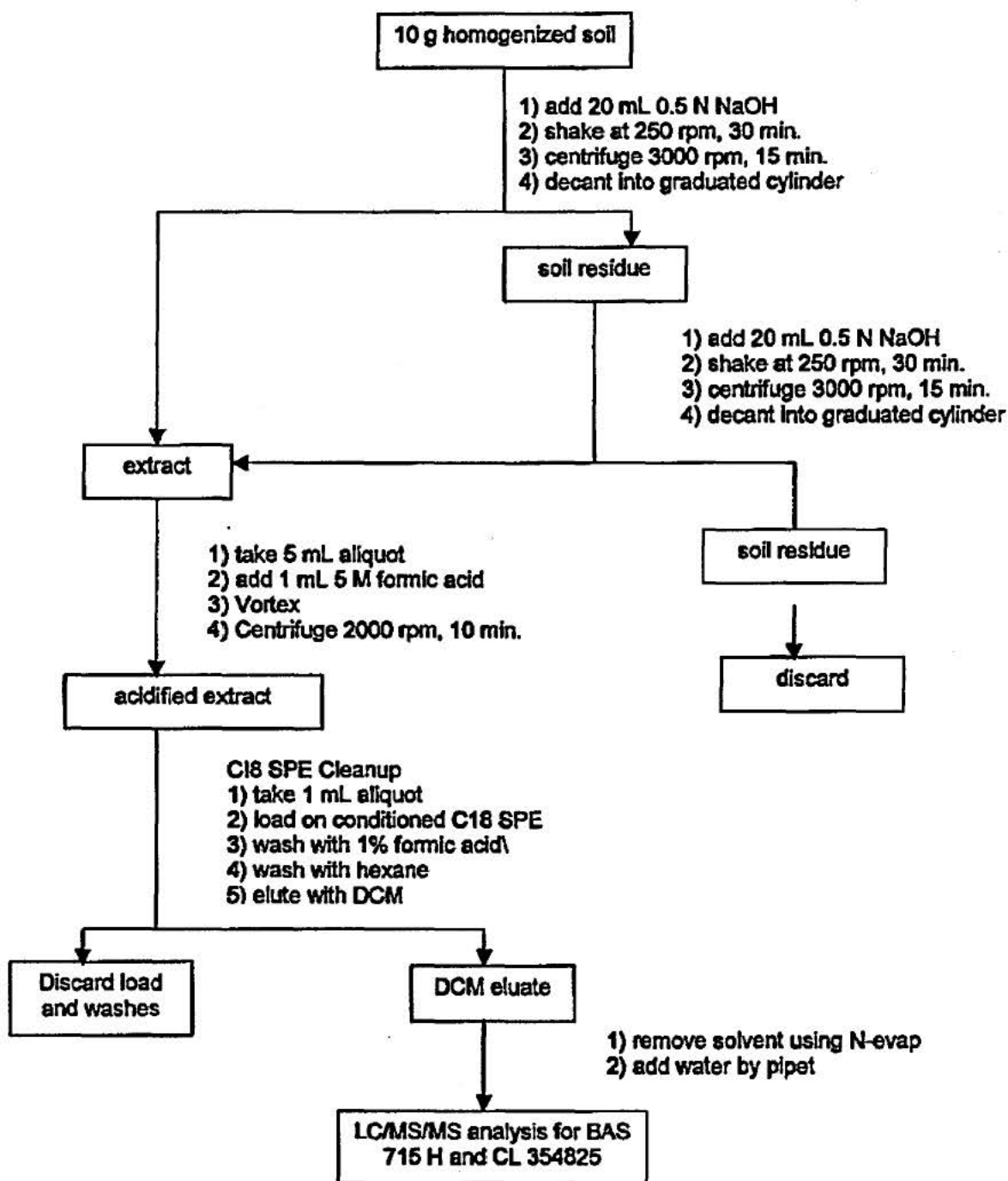


Figure 2 Flow Diagram of Method D0307 with Optional SCX Cleanup

Extraction Procedure is the same as in Figure 1.

