1. INTRODUCTION

1.1 Purpose of the Analytical Phase of Study

The purpose of this study was to demonstrate that BASF Analytical Method D0004/1: "The Determination of Residues of BAS 510 F and Its Metabolites 2-Chloronicotinic Acid and 2-Hydroxy-N-(4'-Chlorobiphenyl-2-yl)Nicotinamide in Soil Using LC-MS/MS" could be performed successfully at an outside facility with no prior experience with the method.

1.2 Summary of Results

The method was successfully validated. The average recoveries obtained for BAS 510 F, 2-Chloronicotinic acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide 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 10x LOQ (0.10 mg/kg) on the first trial.

The recovery results are summarized in **Table 1.**

2. SAMPLE HISTORY

Homogenized control soil, BASF sample code RSN 99513-1861 was received frozen from BASF Corporation, Research Triangle Park, North Carolina on December 05, 2000 and was stored frozen (<-15°C). This soil was identified as a high clay content soil from Illinois and was chosen to represent a more difficult matrix to work with in comparison with other soils. Each validation sample was assigned a unique Battelle identifier associated with laboratory record book 00131.

3. ANALYTICAL METHODS

3.1 Chemistry

The recovery data for the study were generated using BASF Analytical Method D0004/1: "The Determination of Residues of BAS 510 F and Its Metabolites 2-Chloronicotinic Acid and 2-Hydroxy-N-(4'-Chlorobiphenyl-2-yl)Nicotinamide in Soil Using LC-MS/MS". A 10 g soil sample aliquot is extracted with methanol followed by methanol-water, 50:50, v/v. An aliquot (20%) of the extract is diluted with water containing 0.1% formic acid and 4 mM ammonium formate for HPLC-MS/MS determination. A flow diagram of the method is provided in **Figure 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.

3.2 Modifications/Observations for the Method

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

3.2.1 Section 3.5, Instrumentation. The LC-MS/MS conditions differ from the original method due to different instrument parameters available. A Micromass Quattro LC 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 3.4 below.

3.3 Standard Substances and Solutions

Standard solutions for fortification and LC-MS/MS determination were prepared according to method D0004/1 Section 2.4.2. Solvent mix solutions were prepared according the Section 2.3.2.

3.4 LC-MS/MS System

The following conditions, based on Section 3.5 of the supplied method, were used for LC-MS/MS analysis of BAS 510 F, 2-Chloronicotinic Acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide.

HPLC:	Gilson 305 and 306 pumps, Gilson 805 manometric module, Gilson 811C dynamic mixer, and Gilson 235 autosampler
Analytical Column:	Metachem, Intersil ODS-3, 5 µm, 100 x 2.1 mm, part # 0396-100x021
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate

HPLC Gradient:

Time (minutes)	Composition
0.0	20% B
0.1	50% B
1.1	50% B
2.0	70% B
3.0	90% B
4.0	90% B
4.5	20% B
4.6	20% B
7.0	20% B

Flow Rate:	400 µL/minute
Injection Volume:	50 µL
Data Acquisition:	MassLynx, v. 3.2 build 004
Retention Times:	BAS 510 F, ~3.75 minutes
	2-Chloronicotinic acid, ~1.41 minutes
	2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide, ~ 4.17 minutes
Mass Spectrometer:	MicroMass Quattro LC
Ionization:	Positive Ion, APCI (650°C)
Ions Monitored:	m/z 343> 140 (BAS 510 F)
	m/z 158>122 (2-Chloronicotinic acid)
	m/z 325>122 (2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide)

3.5 Data Calculations/Statistical Methods

The concentrations of BAS 510 F, 2-Chloronicotinic acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide 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. 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. The following equation was used to calculate the concentration and percent recovery of each analyte in the samples:

$$\text{Standard Curve: } pg/\mu L = \frac{\text{Peak Area - Intercept}}{\text{Slope}}$$

$$\text{ng Found} = pg/\mu L \times 50\,\mu L \times ng/1000\,pg$$

$$\text{mg Injected} = \frac{\text{Sample Weight (g) extracted}}{\text{Final dilution volume (mL)}} \times \mu L \text{ injected} \times \text{Aliquot Factor}$$

$$\text{Aliquot Factor} = 0.625\ (20\%\ \text{of total extract})$$

$$\text{Calculated Residue (ppm)} = \frac{\text{ng Found}}{\text{mg Injected}}$$

$$\text{Percent recovery (\%)} = \frac{\text{Calculated Residue (ppm) for fortified sample}}{\text{Amount (ppm) fortified}} \times 100$$

Example of Percent Recovery Calculation (Sample No. 00131-16-05, 0.01 ppm Fortification):

Use full computer/calculator precision in any intermediate calculations. Round only the final value.

Curve Statistics: Peak area = m* Concentration + b

slope (m): 34.4760 Intercept (b): -1.24675

$$pg/\mu L\ 2\text{-Chloronicotinic acid} = \frac{77.19 - (-1.24675)}{34.4760} = 2.27511\ pg/\mu L$$

$$\text{ng Found} = 2.27511\,pg/\mu L \times 50\,\mu L \times ng/1000\,pg = 0.1137555\,ng$$

$$\text{mg Injected} = \frac{10\,(\text{g})}{25\,(\text{mL})} \text{ x } 50\,(\mu\text{L}) \text{ x } 0.625 = 12.5\text{ mg}$$

$$\text{Calculated Residue (ppm)} = \frac{0.1137555\text{ ng}}{12.5\text{ mg}} = 0.00910\text{ ppm}$$

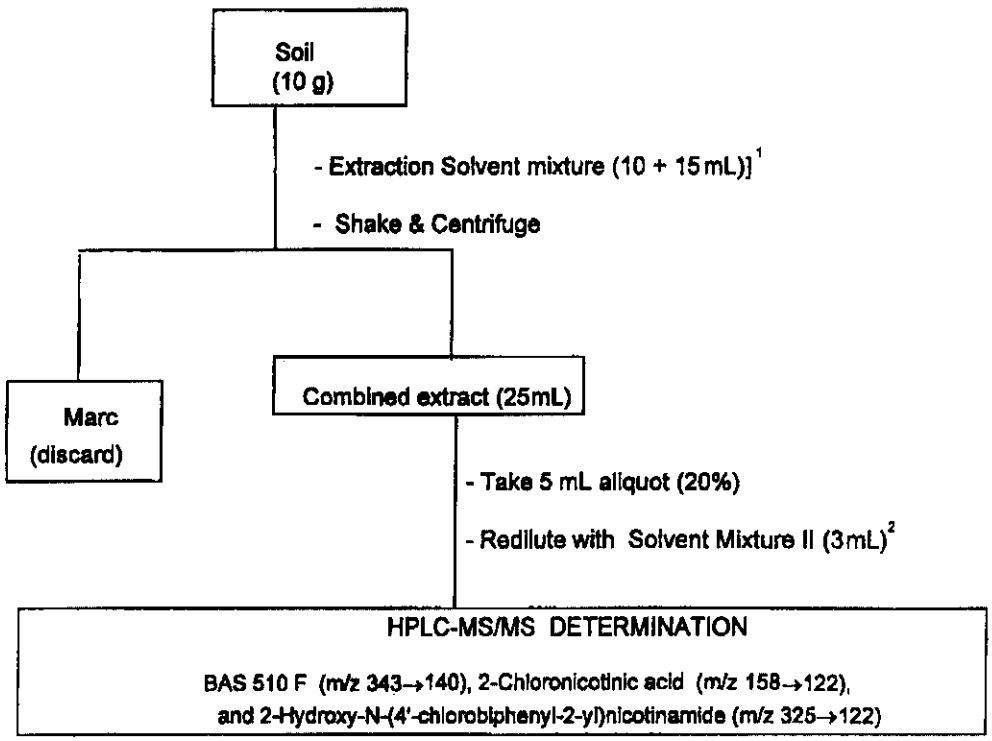
$$\text{Percent Recovery (\%)} = \frac{0.00910\text{ ppm}}{0.010\text{ ppm}} \text{ x } 100 = 91\%$$

Calculated residue (ppm) and percent recoveries of BAS 510 F and 2-Hydroxy-N-(4'-chlorobiphenyl)nicotinamide were calculated in similar fashion.

4.2 Communications with Confirmatory Lab

One communication between the sponsor and the performing laboratory was required during the validation. A discussion of initial low recoveries for the 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide took place. The sponsor suggested several evaluations and changes prior to extracting a second trial. The suggested evaluations confirmed a matrix interference. The HPLC gradient was modified to move the peak away from the interference. The communication was documented in the study file.

Figure 1. Flow Diagram of Method D0004/1

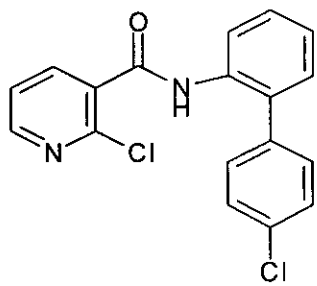


[1] Extraction Solvent Mixture:
First extraction: Methanol
Second extraction: 50:50 Methanol-H2O, v/v

[2] Solvent Mixture II: H2O with 0.1% Formic Acid and 4mM AmmoniumFormate

Figure 2. Description and Structures of Test and Reference Substances

BASF Code Name:	BAS 510 F
BASF Registry Number:	300355
Chemical Name:	2-Chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular Formula:	$C_{18}H_{12}Cl_2N_2O$
Molecular Weight:	343.21
Appearance:	White powder
Water Solubility:	4.63 mg/L
Lot Number:	01183-190
Purity:	99.3 %
Stability:	Expected to be stable at least 2 years
Structural Formula:	



BASF Code Name:	2-Chloronicotinic acid
BASF Registry Number:	107371
Molecular Formula:	$C_6H_4ClNO_2$
Molecular Weight:	157.56
Lot Number:	01174-232
Purity:	99.8%
Stability:	Expected to be stable at least 2 years
Structural Formula:	

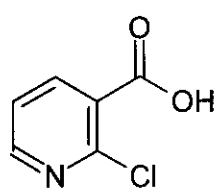


Figure 2. Description and Structures of Test and Reference Substances (Continued)

BASF Code Name:	2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide
BASF Registry Number:	391572
Molecular Formula:	$C_{18}H_{13}ClN_2O_2$
Molecular Weight:	324.77
Lot Number:	01196-217
Purity:	98.9 %
Stability:	Expected to be stable at least 2 years
Structural Formula: