2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Analytical Method # AM-0817 entitled "Determination of Prodiamine and its 6-Amino-Imidazole Metabolite in Soil" (Appendix 1) as performed by Smithers Viscient.

This study was designed to satisfy harmonized guideline requirements described in OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (3).

The residue analytical method is suitable for the determination of prodiamine and 6-amino-imidazole in soil.

Soil recovery samples (20.0 g) were extracted with methanol using a shaker table. Samples were then centrifuged, with an aliquot removed from the supernatant. The aliquot was diluted with 5% sodium chloride solution prior to liquid-liquid extraction using dichloromethane in triplicate. The dichloromethane extract was concentrated followed by reconstitution with 10% ethyl ether in pentane, prior to being stored refrigerated overnight.

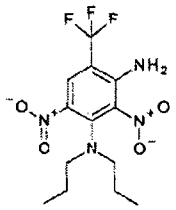
Samples were cleaned using silica gel column chromatography. Prodiamine samples were eluted first followed by concentration using a Kuderna Danish concentrator and reconstituted in toluene. The silica gel column was further rinsed to collect 6-Amino-Imidazole, with this extract concentrated followed by reconstitution in toluene. All samples and standards were analyzed by gas chromatography with micro electron detection (GC/μECD) and gas chromatography with mass spectrometry (GC/MSD).

A summary of the exceptions to the analytical method can be found in Section 3.5.1.

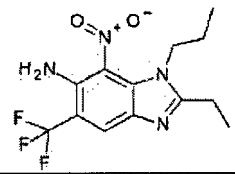
3.0 MATERIALS AND METHODS

3.1 Test Substances

The test substances, prodiamine (SMV No. 6352) and 6-amino-imidazole (SMV No. 6353), were received on 1 August 2013 from Syngenta Crop Protection, LLC, Greensboro, North Carolina. The following information was provided:

Compound Structure	
Syngenta Code:	SAN 745H
Common Name:	Prodiamine
CAS Name:	N^3, N^3-dipropyl-2,4-dinitro-6-(trifluoromethyl)-1,3-benzenediamine
CAS Number:	29091-21-2

Molecular Weight:	350.29
Standard Reference:	DAH-XXIV-52
Storage Conditions:	Refrigerator
Purity:	93.1%
Expiration Date:	30 June 2014

Compound Structure	
Syngenta Code:	SYN 530120
Common Name:	6-Amino-Imidazole
CAS Name:	6-amino-2-ethyl-7-nitro-1-propyl-5-trifluoromethyllbenzimidazole
CAS Number:	Not Listed
Molecular Weight:	316.28
Standard Reference:	ST-IV-33
Storage Conditions:	Refrigerator
Purity:	98.9%
Expiration Date:	30 June 2014

Characterization data for the test substances are maintained by the Sponsor, Syngenta Crop Protection, LLC. The Certificates of Analysis are included in Appendix 2.

The test substances (analytical standards) used in this study were procured from the Sponsor and stored as directed on "Analytical Standards Chain of Custody" documents. All solutions made from the reference substances (analytical standards) were stored according to the method.

3.2 Test System

The test system evaluated in this study was clay loam. This matrix was chosen because it is representative of the matrix the method was designed for. Control sample(s) used in this study were provided by the Sponsor. Control soil sample(s) were characterized by Agvise Laboratories of Northwood, North Dakota and reported to Syngenta Archive under Syngenta Study Number TK0002309, and can be found in Appendix 3. GLP characterization results are presented in Table 1 and summarized below:

Soil Type	pH (0.01M $CaCL_2$)	Sand Content (% w/w)	Silt Content (% w/w)	Clay Content (% w/w)	Organic Matter (%)
Clay Loam	6.0	25	43	32	4.2

Note: The GLP characterization of these soil types was performed by Agvise Laboratories, 604 Highway 15, P.O. Box 510, Northwood, ND 58267.

The soil was received from Syngenta Crop Protection, Greensboro, North Carolina on 4 October 2013. The soil was stored at ambient temperatures prior to testing.

These control sample(s) were checked for contamination prior to use in this ILV study by employing the same extraction and detection methods as described in the analytical method, Method # AM-0817.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method.

3.3.1 Equipment

1.	Instrument:	Hewlett Packard Series 7890A gas chromatograph equipped with a Hewlett Packard Series 7890A micro electron capture detector (μECD), a Hewlett Packard Series 7890A autosampler, a Hewlett Packard Series 7890A injector and Agilent Chemstation ECM version B.04.03 Software for data acquisition Hewlett Packard Series 6890 gas chromatograph equipped with a mass selective detector (MSD) Series 5973, Hewlett Packard autosampler, Hewlett Packard Series 7683 injector and Agilent Chemstation ECM version E.02.02 Software for data acquisition.
2.	Balances:	Mettler AG240, Mettler PJ3000, Sartorius Moisture Analyzer MA-45
3.	Laboratory equipment:	Positive displacement pipets, disposable glass pipets, volumetric flasks, 8 oz. amber glass bottles, orbital shaker table, drying oven, separatory funnels, round bottom flasks, Kuderna Danish concentrator set-up (with distillation receiver and Vigreaux condenser), autosampler vials, Teflon®-lined caps.
5.	Centrifuge:	Beckman Model Allegra X-12

3.3.2 Reagents

1.	Silica gel 60 (70-230 mesh):	Alfa Aesar, reagent grade
2.	Dichloromethane	EMD, reagent grade
3.	Ethyl ether (2% EtOH preservative	Burdick and Jackson, reagent grade
4.	Sodium sulfate, anhydrous:	EMD, reagent grade
5.	Sodium chloride:	BDH, reagent grade

6.	Methanol:	EMD, reagent grade
7.	Pentane:	BDH, reagent grade
8.	Toluene:	EMD, reagent grade
9.	Hexane:	EMD, reagent grade
10.	Purified reagent water:	prepared from a Millipore Milli-Q® Direct 8 system (meeting ASTM Type II requirements)

3.3.3 Preparation of Reagents

A 5% sodium chloride in purified reagent water liquid reagent solution was typically prepared by dissolving 50.0 g of sodium chloride in 1000 mL of purified reagent water. The solution was mixed well using a stir bar and stir plate.

A 10% ethyl ether in pentane liquid reagent solution was typically prepared by combining 200 mL of ethyl ether with 1800 mL of pentane and mixed well using a stir bar and stir plate.

A 50% ethyl ether in pentane liquid reagent solution was typically prepared by combining 500 mL of ethyl ether with 500 mL of pentane and mixed well using a stir bar and stir plate.

A 3% water deactivated silica gel reagent was typically prepared by spreading a one inch deep layer of 485 g of silica gel in a glass dish and was then activated at 250 °C for 25 hours. The solution was cooled in a tightly capped bottle and 15 g of deionized water was added to achieve 3% deactivation of the silica gel. The reagent was placed on a shaker table overnight at 150 rpm.

3.4 Preparation of Standard Solutions

All primary and secondary stock solutions were stored refrigerated in glass amber bottles fitted with Teflon®-lined caps. All sub-stock solutions were prepared daily and discarded after use.

3.4.1 Stock Standards

A 1.00 mg/mL (1000 ng/µL) primary stock solution of prodiamine was typically prepared by placing approximately 0.0269 g of test material (0.0250 g as active ingredient) in a volumetric flask and bringing it to volume with 25.0 mL of toluene.

A 1.00 mg/mL (1000 ng/µL) primary stock solution of 6-amino-imidazole was typically prepared by placing approximately 0.0253 g of test material (0.0250 g as active ingredient) in a volumetric flask and bringing it to a volume of 25.0 mL with toluene.

3.4.2 Fortification Standards

Three fortification stock solutions (1.00, 10.0 and 100 ng/µL) of prodiamine were prepared by placing 0.0500, 0.500 and 5.00 mL of the 1000 ng/µL primary stock solution in separate

volumetric flasks and bringing each to a volume of 50.0 mL with toluene. The 1.00 ng/µL fortification stock solution was used to prepare the matrix matched standards used for the matrix effect analysis. The 10.0 and 100 ng/µL fortification stock solutions were used to prepare mixed fortification stock solutions.

Three fortification stock solutions (1.00, 10.0 and 100 ng/µL) of 6-amino-imidazole were prepared by placing 0.0500, 0.500 and 5.00 mL of the 1000 ng/µL primary stock solution in separate volumetric flasks and bringing each to volume with 50.0 mL of toluene. The 1.00 ng/µL fortification stock solution was used to prepare the matrix matched standards used for the matrix effect analysis. The 10.0 and 100 ng/µL fortification stock solutions were used to prepare a mixed fortification stock solutions.

A 1.00 ng/µL mixed fortification stock solution was typically prepared by combining 1.00 mL of the 10.0 ng/µL prodiamine fortification stock solution with 1.00 mL of the 10.0 ng/µL 6-amino-imidazole fortification stock solution and bringing it to a final volume of 10.0 mL with toluene. This mixed fortification stock solution was used to prepare the low-level recovery samples and calibration standards.

A 10.0 ng/µL mixed fortification stock solution was typically prepared by combining 1.00 mL of the 100 ng/µL prodiamine fortification stock solution with 1.00 mL of the 100 ng/µL 6-amino-imidazole fortification stock solution and bringing it to a final volume of 10.0 mL with toluene. This mixed fortification stock solution was used to prepare the high-level recovery samples and calibration standards.

All primary and fortification stock solutions were stored refrigerated in glass amber bottles fitted with Teflon®-lined caps. All mixed fortification stock solutions were prepared daily and discarded after use.

3.4.3 Calibration Standards

Calibration standards were prepared in toluene at concentrations of 0.00500, 0.00750, 0.0100, 0.0200 and 0.0500 ng/µL using the 1.00 ng/µL mixed fortification stock solution and at concentrations of 0.100, 0.500 and 1.00 ng/µL using the 10.0 ng/µL mixed fortification stock solution.

3.4.4 Matrix-Matched Standards

Due to the possible matrix interference from the soil, an additional analysis comparing the soil matrix to the solvent (toluene) was conducted. Control matrix matched standards and solvent standards were prepared in triplicate at a concentration of 0.0100 ng/µL from the 1.00 ng/µL prodiamine or 6-amino-imidazole fortification stock solution.

3.5 Analytical Procedures and Modifications

Analytical Method # AM-0817 was independently validated as written except for the method modifications described in Section 3.5.1.

3.5.1 Modifications

Syngenta Crop Protection, LLC Analytical Method # AM-0817 was followed as written with the following exceptions:

- The original method stated that silica gel 60 (70 – 200 mesh) would be used. This silica gel is no longer available for purchase and therefore silica gel 60 (70 – 230 mesh) was substituted.
- The original method did not indicate a shaker table speed, therefore all samples were placed on a shaker table at 150 rpm.
- The original method stated that the samples would be concentrated to 1.0 mL using a water bath at 60 °C. All samples were concentrated to 1.0 mL using a water bath at 56 °C, as that was the maximum temperature achievable for the equipment.
- The original method stated that 100 mL of 50% ethyl ether in pentane would be used to further elute the column during the 6-amino-imidazole process, and would be discarded. During the silica gel profile conducted prior to experiment initiation, results indicated test material losses were occurring during this rinse step. Therefore, the amount of 50% ethyl ether in pentane was increased to 200 mL and combined with the 100 mL elution of ethyl ether in an attempt to capture all 6-amino-imidazole during extraction.
- The original method stated that for GC/μECD conditions, the oven temperatures would have an initial value of 185 °C, with an initial time of 6 minutes and a post value of 225 °C with a post time of 5 minutes. The retention times were 2.95 and 4.56 minutes in the original method for prodiamine and 6-amino-imidazole, respectively. During the ILV, test substance retention times differed from the original analysis, yielding ~5.6 and ~8.7 minutes for prodiamine and 6-amino-imidazole, respectively. If the initial time was left as 6 minutes from the original method, 6-amino-imidazole was being lost. Therefore, the initial time and post time were combined; the run time was updated to 12 minutes (which includes the 5 minute post run) so that 6-amino-imidazole could be detected.
- The original method stated that for GC/MSD conditions, the oven temperature would contain the following:

Profile:	Level 1
Program Rate:	30 °C/minute
Final Value:	190 °C
Final Time:	5.5 minutes
Post Value:	250 °C
Post Time:	5 minutes
Retention Times:	7.25 minutes for prodiamne 8.11 minutes for 6-amino-imidazole

- During the ILV, test substance retention times differed from the original analysis, yielding approximately 9.6 and 10.4 minutes for prodiamine and 6-amino-imidazole, respectively. When utilizing the five minute post time from the original method, the run was not detecting prodiamine or 6-amino-imidazole (as they were eluting too late). The post time was combined with the run time so that the entire spectra could be collected: Rate: 30 °C, Final Temperature: 190 °C, Final Time: 5.5 minutes, Rate 30 °C, Final Temperature: 250 °C, Final Time: 5.0 minutes. No post temperature or post time was utilized.

3.5.2 Fortifications

Untreated control soil samples were fortified using microliter amounts of the appropriate fortification standard to LOQ and 10X LOQ concentrations as per method.
Fortifications used in this method validation are as follows:

Matrix	Fortification Concentration (ng/µL)	Fortification Volume (mL)	Sample Dry Weight (g)	Final Concentration (ppm)	Replicates (n)
Clay Loam	1.00	0.200	20.0	LOQ (0.0100)	5
Clay Loam	10.0	0.200	20.0	10X LOQ (0.100)	5

Two additional 20 g soil samples (per sample set) were prepared and left unfortified to serve as the controls. One additional sample was extracted using only extraction solvents to serve as the reagent blank.

Following fortification, each recovery sample was allowed to stand for 15 minutes to allow the spiking solution to evaporate prior to the extraction procedure. Samples were then vortex mixed.

3.5.3 Analytical Procedure

A summary of the Method # AM-0817 is described below:
A 200 mL aliquot of methanol was added to each sample and placed on a shaker table at 150 rpm for 30 minutes. Samples were then centrifuged for 30 minutes at 550 G and the supernatant was decanted into 8.0-oz.amber bottles. A 50.0 mL aliquot was then transferred to a 500-mL separatory funnel containing 250 mL of a 5% sodium chloride solution and 25 mL of dichloromethane. Samples were shaken for one minute and the dichloromethane layer was drained through a glass funnel with a small plug of anhydrous sodium sulfate into a 250-mL round bottom flask. The dichloromethane extraction procedure was repeated twice for a total of three times. The extracts were combined and placed into a 250 mL round bottom flask. The sodium sulfate was washed three times with 5.0 to 10 mL of dichloromethane and the rinse collected into the appropriate 250 mL round bottom flask. The dichloromethane extracts were then taken to near dryness using a rotary evaporator in a 40 °C water bath. The remaining dichloromethane was taken to dryness under a gentle

stream of nitrogen. The samples were then reconstituted with 5.0 mL of 10% ethyl ether in pentane and stored refrigerated overnight, covered with foil to protect from ultraviolet lights.

A 70 mL aliquot of 10% ethyl ether in pentane was added to a 250 mL separatory funnel and 20 g of the 3% of water deactivated silica gel was slowly added. The separatory funnel was shaken well and quickly drained into a chromatographic column plugged with glass wool. The separatory funnel was rinsed with 10 mL of 10% ethyl ether in pentane and added to the chromatographic column. Granular sodium sulfate (1.0 cm) was added to the column once the silica gel had completely settled and the solvent was drained to just above the top of the sodium sulfate layer. The 5.0 mL aliquot of 10% ethyl in pentane sample solution was added to the silica gel column and again was allowed to drain to just above the top of the sodium sulfate layer. The 250 mL round bottom flasks were then rinsed twice with 5.0 mL portions of 10% ethyl ether in pentane, adding the rinse to the silica gel column each time, and allowing each rinse to drain to just above the sodium sulfate layer. An additional 70 mL of 10% ethyl ether in pentane was passed through the column and was then discarded.
A 75 mL aliquot of 10% ethyl ether in pentane was used to elute prodiamine into a Kuderna Danish concentrator with a 15-mL distillation receiver attached, and 1 mL of hexane added to the sample. A Vigreaux condenser was connected to the Kuderna Danish set-up and the sample was concentrated to approximately 1.0 mL in 56 °C water bath in a fume hood. The remaining solvent was evaporated to dryness under a gentle stream of nitrogen. A 5.0 mL aliquot of toluene was added to the 15-mL distillation receiver to thoroughly dissolve the residue. This final sample was analyzed for prodiamine.

The silica gel column was further rinsed with 200 mL of 50% ethyl ether in pentane and the rinse collected in a 500-mL round bottom flask. A 100 mL aliquot of ethyl ether was used to elute 6-amino-imidazole into the 500-mL round bottom flask. The solution was taken to near dryness using a rotary evaporator in a 40 °C water bath. The remaining solvent was taken to dryness under a gentle stream of nitrogen. A 5.0 mL aliquot of toluene was added to the round bottom flask to thoroughly dissolve the residue. This final sample was analysed for 6-amino-imidazole.

A typical sample preparation for both prodiamine and 6-aminio-imidazole is described below.

Sample ID	Nominal Concentration (ppm)	Sample Weight (g)	Extraction Volume[a] (mL)	Sample Aliquot Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Reagent Blank	0.00	NA[c]	200	50.0	5.00	1.00
Control C, D, E and F	0.00	20.0	200	50.0	5.00	1.00
Low E, F, G, H and I	0.0100	20.0	200	50.0	5.00	1.00
High A, B, C, D and E	0.100	20.0	200	50.0	5.00	1.00

[a] Extraction solvent: Methanol.
[b] Sample diluent: Toluene.
[c] NA = Not Applicable.

3.6 Instrumentation

The gas liquid chromatographic analysis with micro electron capture detection (GC/μECD) was conducted utilizing the following instrumental conditions:

Column:	Agilent HP-17, 10 m x 0.53 mm x 2.0 μm
Gas flows:	Carrier gas: Helium held at a constant pressure of 6 psi
Detector make-up gas:	Argon/methane (5%) at a combined flow 18 mL/min
Temperatures:	Injector: 250 °C
	Detector: 350 °C
Oven:	Initial temperature: 185 °C
	Initial time: 12 min
Flow rate:	14.017 mL/min
Injection Volume:	2.00 μL
Inlet mode:	Splitless (purge flow on at 60 mL/min at 0.50 min)
Retention Time:	approximately 5.6 minutes for prodiamine
	approximately 8.7 minutes for 6-amino-imidazole

The gas chromatographic analysis with mass selective detection (GC/MSD) was conducted utilizing the following instrumental conditions:

GC Parameters:

Column: Agilent HP-1, 25 m × 0.20 mm × 0.11 μm

Temperature: 100 °C (initial) and held for 0.50 minute

Ramps:

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
30.0	190	5.50
30.0	250	5.00

Run Time:	16 minutes
Injection Volume:	2.0 μL
Gas Flows:	Carrier Gas: helium, at a constant pressure of 5 psi
Flow rate:	0.3 mL/min
Inlet Mode:	Splitless
	Purge Time: on at 0.50 minute at 60.0 mL/min
	Inlet Temperature: 250 °C
Retention Time:	approximately 9.6 minutes for prodiamine
	approximately 10.4 minutes for 6-amino-imidazole

MSD Parameters:

Solvent Delay:	3.00 minutes
MSD Source Ionization:	electron impact

Selected Ion Monitoring:

Transition Ions (m/z)	Test Substance/Transition	Dwell (msec)
321	Prodiamine (primary)	50
279	Prodiamine (confirm #1)	50
333	Prodiamine (confirm #2)	50
316	6-Amino-Imidazole (primary)	50
228	6-Amino-Imidazole (confirm #1)	50
239	6-Amino-Imidazole (confirm #2)	50

Temperatures:

MSD Transfer Line: 250 °C
MS Source: 230 °C, maximum 250 °C
MS Quad: 150 °C maximum 200 °C

3.7 Data Acquisition

Peak integration and peak area count quantitation were performed by Agilent ChemStation software ECM (version E.02.02 for GC/MSD or version B.04.03 for GC/μECD). A quadratic equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of analyte. The square of the correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99. Recovery results were computed for each sample.

A statistical treatment of the data includes the calculation of averages, standard deviations and relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using the current version of Microsoft Office Excel.

Independent Laboratory Validation (ILV) of the Analytical Method: Determination of Prodiamine and its 6-Amino-Imidazole Metabolite in Soil (Method # AM-0817)

1.0 INTRODUCTION

The purpose of this study is to confirm that an analytical method, developed by one group, can be independently validated by a second group in the absence of major interaction between the two. This study is required by EPA under Guideline OCSPP 850.6100 (2012): Environmental Chemistry Methods and Associated Independent Laboratory Validation [EPA 712-C-001] and Guideline OCSPP 850.7100: Data Reporting for Environmental Chemistry Methods [EPA 712-C-96-348]. *Independent labs are allowed to analyze three sample sets* in order to validate the method as written. A complete set of samples should consist of, at a minimum, two un-spiked matrix control samples, five matrix control samples fortified at the limit of quantification (LOQ), and five matrix control samples fortified at 10X LOQ for each distinct matrix. A complete set may include more than twelve samples depending on the number of reagents, and un-fortified and fortified control matrix samples. It may be necessary, however, to divide a complete set into two subsets for efficient handling. Each subset should contain a two un-fortified matrix control samples and five matrix control samples fortified at the LOQ or 10X LOQ.

If the performance data on the first set of samples at any of the required spiking levels is unsuccessful, the independent laboratory may contact the registrant to clarify the directions given in the method. Any contact with the registrant or developers during the method validation must be documented in writing in the final report submitted by the independent laboratory. If the independent laboratory cannot generate performance data that is similar to the registrant's or developers' after the second set of spiked samples, the independent laboratory may contact the registrant to further clarify the directions given in the method. If the independent laboratory cannot generate performance data that is similar to the registrant's or developers' after the third set, the method should be failed and a report will be sent to the registrant explaining why the method failed. The registrant should then decide whether to repeat the independent laboratory validation at another laboratory, further develop the method or withdraw it. This ILV trial will be conducted under FIFRA Good Laboratory Practice (GLP) standards as specified in 40 CFR part 160. A maximum of three sample sets are used by an independent laboratory to validate the method as written. A successful ILV trial will require adequate results on at least one complete set of samples on a given matrix.

The purpose of this protocol is to perform an ILV for the analytical method used to determine the test substance in up to three soil types. The analytical method will be validated with regards to accuracy, precision, signal response, selectivity, and limits of quantitation. The method to be validated is attached to this protocol as Appendix I.

Additional validation testing will be performed to check for matrix effects at the LOQ level for both analytes. The matrix effect procedure should be approved by sponsor study monitor prior to execution.

2.0 OBJECTIVE

Syngenta Analytical Method AM-0817 (Reference 1) is suitable for the determination of Prodiamine and its 6-Amino-Imidazole Metabolite in soil. The limit of quantitation (LOQ) of the method has been established at 0.01 ppm. The objective of this study is to perform an independent laboratory validation of the analytical method using a representative soil sample.

Additional validation testing will be performed to check for matrix effects at the LOQ level for both analytes. The matrix effect procedure should be approved by sponsor study monitor prior to execution.

3.0 JUSTIFICATION OF THE TEST SYSTEM

The method validations described in this protocol are designed to conform to EPA Guideline OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation [EPA 712-C-001] and Guideline OCSPP 850.7100: Data Reporting for Environmental Chemistry Methods [EPA 712-C-96-348].

The control samples will be analyzed with the method for evaluation of substrate-related interferences, and the fortified samples will be analyzed using the method for evaluation of method performance via procedural recoveries.

4.0 MATERIALS

4.1 Test Substances

The test substances will be supplied by the registrant, Syngenta. Upon arrival at Smithers Viscient, the test substance(s) will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test substances will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

Each test substance will be given a unique sample ID number and stored under the conditions specified by the Sponsor or manufacturer. The following information should be provided by the Study Sponsor, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

Test solution preparation will be documented on data forms which include (but not limited to) the amount of test substance, the volume or mass of the test solution, lot, batch or other sample designation of the test substance and date the solution was prepared. Individual sample containers will be labeled with a unique ID number.

4.2 Reagents

Sources for the solvents and chemical reagents are giving in the analytical method. If equivalent materials are substituted for the specified materials, the source and part number of each will be recorded in the study records.

5.0 TEST SYSTEM IDENTIFICATION

The test system for this study will consist of a soil sample type based on a difficult matrix for which this method will be used. Untreated control samples (UTC) will be provided by the Study Sponsor for independent laboratory validation of the analytical method (Appendix 1) along with the characterization information. Control samples should be stored frozen prior to analysis.

SAMPLE	MATRIX	SAMPLE DESCRIPTION
Underwood Farm 0-6"	Soil	Clay Loam

6.0 ANALYTICAL METHOD

The analytical method "AM-0817-Determination of Prodiamine and its 6-Amino-Imidazole Metabolite in Soil" is attached in Appendix I.

7.0 VALIDATION DESIGN

Prior to conducting the ILV, the performing laboratory will need to establish method control not limited to but including analyte retention time, linearity, instrument response, instrument detection limits, procedures and verification that the control soil matrix is free of interferences. The performing laboratory should demonstrate method control by performing assessment tests before proceeding to method validation trials. More than one assessment test may be made depending on the number and type of substitutions. Data and results of any assessment test shall be included in the study records, but not in the final report.

Control soil samples will be fortified with known amounts of Prodiamine and its 6-Amino-Imidazole Metabolite and analyzed using the procedures outlined in analytical method (Appendix 1).

Validation Set:

1x Reagent Blank (matrix free sample submitted to procedures outlined in method)

2x Control Soil (untreated control soil)

5x Control Soil + LOQ (mg/kg) (5 replicates at the Target LOQ)

5x Control Soil + 10X LOQ (mg/kg) (5 replicates at 10X the Target LOQ)

The independent laboratory should verify that the matrix control materials are free of interferences at the appropriate retention time or detector setting by examining the control samples under the instrumental conditions specified in the method. A response greater than

30% of each proposed LOQ constitutes a significant interference. If this is observed, the Study Monitor will be contacted for direction on how to proceed.

The standard curve will be comprised of at least five concentrations. The anticipated concentration range is approximately 0.01 – 1.0 mg/L. A smaller, larger, or shifted range may be necessary if achievable. The range will be documented in the study records and final report.

The limit of detection (LOD) will be established by evaluating the signal-to-noise (S/N) ratio from samples of known concentration and blank samples to establish the lowest level at which the analyte can be reliably detected. A S/N ratio of 3:1 is generally considered the minimum acceptable ratio for reliable detection.

Additional validation testing will be performed to check for matrix effects at the LOQ level for both analytes. The matrix effect procedure should be approved by sponsor study monitor prior to execution.

7.1 Accuracy and Precision

The accuracy of the analytical method will be determined by applying the method to five samples of soil at the LOQ (0.01 ppm) and five samples at 10X LOQ (0.10 ppm) for Prodiamine and its 6-Amino-Imidazole Metabolite . The accuracy will be reported in terms of percent recovery and the difference between the mean determined and the theoretical value. Recoveries of 70 to 120% of nominal are acceptable.

The precision will be calculated for the fortified samples in terms of the mean, range, standard deviation (SD) and relative standard deviation (RSD or coefficient of variation (CV)) calculated for the retention time, peak area based quantitation (i.e., µg/L), and the observed recovery values. The retention time should have a RSD of less than or equal to 2%. The RSD of the peak area based quantitation (i.e., µg/L) should be less than or equal to 20%. The RSD of the recovery values should be less than or equal to 20% as well.

7.2 Specificity

The specificity of the method will be determined by applying the method to two un-fortified matrix control samples for each matrix. Chromatograms will be obtained for the control samples and examined for peaks that might interfere with the quantitation of the analyte peak of interest. Peaks attributable to test substance should be sufficiently resolved from any peaks found in the samples of control matrix to enable quantification. Interferences with peak areas that are less than 30% at the limit of detection (LOD) are not considered significant.

7.3 Signal Response

The signal response of the method will be determined by preparing a calibration curve with a minimum of five standards to encompass 70 to 120% of the test concentration.

The calibration data will be subjected to a regression analysis; a plot of the analyte concentration versus the detector response will be included in the report along with the correlation coefficient, y-intercept, and slope of the regression line. The signal response data should have an intercept close to zero and a correlation coefficient (r^2) not less than

0.990. The responses of the standards shall be inserted into the regression equation, and a calculated concentration value calculated. This calculated value shall be within ±20% of the theoretical value. Deviations from these criteria will be addressed by reevaluating the calibration range, such that the calculated values meet these criteria.

8.0 CONTROL OF BIAS

Bias will be effectively controlled by experimental design and statistical methods through techniques such as, but not limited to, preparation of replicate samples, replicate analysis, procedural recoveries from a homogeneous mixture by fortification, and maintenance of material balance.