I. SUMMARY

EN-CAS Analytical Laboratories conducted a successful independent laboratory validation of a method for the determination of SL-160 and its metabolites (TPSA, DTPU, DTPP, and ADMP) in soil. The method was authored by CCRL (CCRL-MTH-045, see Appendix I). The study was conducted under EN-CAS Protocol No. 03-0026 (see Appendix II).

The validation trial consisted of two subsets. Subset 1 included one reagent blank, one unfortified control sample, three fortifications at the Limit of Quantitation (LOQ, 2.5 ppb) and two fortifications at 25 ppb with each of the five analytes. Subset 2 included one unfortified control sample, two fortifications at the Limit of Quantitation (LOQ, 2.5 ppb) and three fortifications at 25 ppb with each of the five analytes.

The first trial was successful in that the results achieved for all the analytes were similar to those achieved by the method developers. One analyte, ADMP, gave mean recoveries below the 70-120% range. This is similar to results achieved by CCRL for this analyte.

II. OBJECTIVE

The purpose of this study was to perform an independent laboratory validation (ILV) of CCRL's Method CCRL-MTH-045, "Analytical Method for the Determination of SL-160 and Its Metabolites in Soil" (Appendix I) to satisfy requirements described in the EPA Guideline, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods.

III. INTRODUCTION

The EPA Guideline, OPPTS 850.7100, includes a requirement for registrants to validate analytical methods for the determination of residues in soil at an independent laboratory prior to submission to the EPA. This report details the results of the confirmatory trial of CCRL's Method CCRL-MTH-045, (Appendix I), authored by Megan Stockton, CCRL, Fresno, CA. The study was conducted according to EN-CAS Protocol No. 03-0026 entitled "Independent Laboratory Validation (ILV) of CCRL'S Method (CCRL-MTH-045) for the Analysis of SL-160 and Its Metabolites (TPSA, DTPU, DTPP, and ADMP) in Soil", included as Appendix II to this report.

As described in the protocol, the validation trial consisted of two subsets. Subset 1 included one reagent blank, one unfortified control sample, three fortifications at the Limit of Quantitation (LOQ, 2.5 ppb) and two fortifications at 25 ppb with each of the five analytes. Subset 2 included one unfortified control sample, two fortifications at the Limit of Quantitation (LOQ, 2.5 ppb) and three fortifications at 25 ppb with each of the five analytes.

The study was initiated on November 6, 2003. The experimental start date was November 24, 2003 and the experimental termination date was November 26, 2003. Standard solutions were prepared before the experimental start date to be available for instrument setup. The preparation, storage, and accompanying documentation was in compliance with the method, EN-CAS SOP's, and the GLP regulations.

IV. TEST SYSTEM

Control soil used in the validation study was received (frozen) on November 6, 2003 from CCRL, Fresno, CA. The sample was assigned an EN-CAS ID number of ES2364. The sample was stored in a freezer at a temperature of -10 °C or lower. The soil sample was removed from the freezer only for weighing subsamples and then immediately returned after use. Sample log-in information can be found in the raw data package associated with this study. Sample storage records are on file at EN-CAS Analytical Laboratories.

V. TEST AND REFERENCE MATERIALS

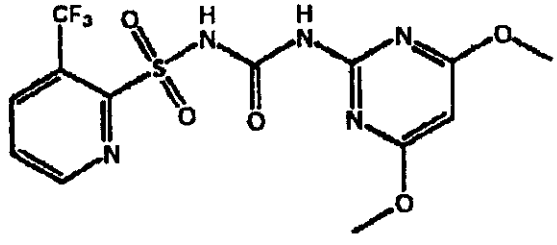
Analytical grade SL-160, TPSA, DTPU, DTPP, and ADMP standards were received at EN-CAS on November 11, 2003 from Midwest Research Institute, Kansas City, MO and were used for preparation of stock, fortification, and calibration standards. The standards were received frozen and were stored under

freezer conditions (approximately -10 °C). Characterization of the test/reference materials was performed by Midwest Research Institute, Kansas City, MO, which retains the characterization data on file. The Certificates of Analysis of the test/reference materials can be found in Appendix III.

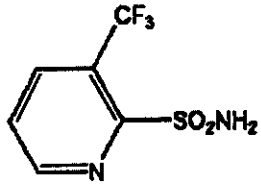
The following information accompanied the test/reference materials upon receipt at EN-CAS.

Standard Reference	EN-CAS Number	% Purity	Lot/Batch Number	Expiration Date	Physical Appearance
SL-160	ES2355	99.89	Y-920205	12/13/06	White powder
TPSA	ES2359	100	0205	6/13/07	Light powder
DTPU	ES2358	98.4	0205	6/13/07	Light powder
DTPP	ES2357	98.4	0205	6/13/07	Light powder
ADMP	ES2356	99.9	Y-Ba.79	6/13/07	Light powder

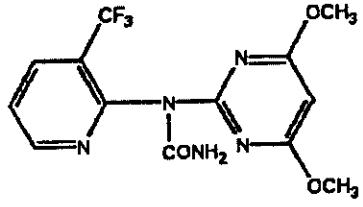
Common Name: SL-160, Flazasulfuron
IUPAC Chemical Name: 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulphonyl)urea
CAS Chemical Name: *N*-[[4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(trifluoromethyl)-2-pyridinesulfonamide
CAS No.: 104040-78-0
Empirical Formula: $C_{13}H_{12}F_3N_5O_5S$ (MW=407.3)
Chemical Structure:



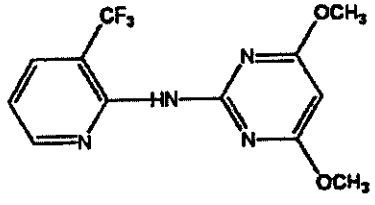
Common Name: TPSA
IUPAC Chemical Name: 3-trifluoromethyl-2-pyridinesulphonamide
CAS No.: Not available
Empirical Formula: $C_6H_5F_3N_2O_2S$ (MW=226.18)
Chemical Structure:



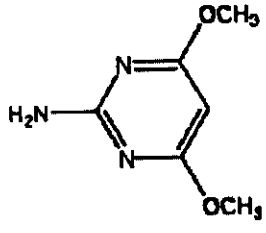
Common Name: DTPU
IUPAC Chemical Name: 1-(4,6-dimethoxypyrimidin-2-yl)-1-(3-trifluoromethyl-2-pyridyl)urea
CAS No.: Not available
Empirical Formula: $C_{13}H_{12}F_3N_5O_3$ (MW=343.26)
Chemical Structure:



Common Name: DTPP
IUPAC Chemical Name: 4,6-dimethoxy-2-(3-trifluoromethyl-2-pyridylamino)pyrimidine
CAS No.: Not available
Empirical Formula: $C_{12}H_{11}F_3N_4O_2$ (MW=300.24)
Chemical Structure:



Common Name: ADMP
IUPAC Chemical Name: 2-amino-4,6-dimethoxypyrimidine
CAS No.: Not available
Empirical Formula: $C_6H_9N_3O_2$ (MW=155.15)
Chemical Structure:



VI. DESCRIPTION OF ANALYTICAL METHOD

CCRL Laboratory's Analytical Method CCRL-MTH-045, "Analytical Method for the Determination of SL-160 and Its Metabolites in Soil" (attached as Appendix I) was used for this study. The method is designed to determine individual residues of SL-160 and its metabolites, TPSA, DTPU, DTPP, and ADMP, in soil. Procedural recoveries are separately determined for SL-160, TPSA, DTPU, DTPP, and ADMP by fortifying control samples with a combined standard containing all five analytes.

SL-160, TPSA, DTPU, DTPP, or ADMP was extracted from the matrix by shaking a 50-g soil sample with 120 mL of acetonitrile (MeCN) and 30 mL water (H_2O) for 30 minutes at approximately 200 rpm on a reciprocating shaker. The sample was then filtered through a Buchner funnel and reduced to 10-20 mL by vacuum rotary evaporation with a bath temperature of approximately 40 °C. The sample residue was dissolved in additional H_2O and extracted with methylene chloride (DCM). Following the DCM partition, the aqueous layer was acidified with 6N HCl and partitioned again with DCM. The combined DCM fractions were reduced to dryness and reconstituted with MeCN, filtered and transferred to a vial for LC/MS/MS analysis.

The following minor adjustment was made to the method.

1. The volume of DCM was slightly increased to allow the use of available laboratory equipment. A 50-mL Tilt-a-pet was used as a delivery system when a 40-mL volume was requested. Since the DCM was then reduced to dryness, the total volume was immaterial.

VII. EXPERIMENTAL DESIGN

A. Establish Method Chromatography and Performance Criteria

1. Preliminary Method Setup

Prior to performing the ILV, EN-CAS determined approximate analyte retention times and instrument detection limits for SL-160 and its metabolites. Linear calibration curves were established by injecting standards at five levels ranging from 0.001 µg/mL to 0.020 µg/mL.

2. Preparation of Stock, Fortification and Calibration Standards

Individual stock standard solutions of SL-160 and the four metabolites (1000 µg/mL in MeCN) were prepared on 11/17/03.

Aliquots of the stocks were mixed and diluted with MeCN to prepare 1.00 µg/mL and 10.0 µg/mL fortification solutions.

Appropriate aliquots of the 10.0 µg/mL solution were further diluted in MeCN to generate a series of calibration standards containing 0.0010, 0.002, 0.005, 0010, and 0.020 µg/mL, respectively.

All solutions were stored in a freezer at approximately -10 °C. All standard solutions were stored in amber bottles. Further information regarding the preparation of fortification standards and HPLC calibration standards is located in the file for EN-CAS Study No. 03-0026.

3. Calibration Curve

Standards were injected at the beginning, at the end, and interspersed throughout each run at the following levels: 0.001, 0.002, 0.005, 0.010, and 0.020 µg/mL. The calibration curve used was a linear regression curve, $y = mx + b$, where m is the slope and b is the y-intercept. A validated Excel spreadsheet was used to calculate the data. Example calibration curves from subset 1-01-MV are shown in Figure 7.

4. Chromatography

The control matrix was free of significant interference at the various analyte retention times. Example chromatograms of standards, controls, and fortified samples are shown in Figures 1 - 6.

5. Description of Instrument and Operating Conditions

The HPLC instrumentation and operating conditions are as follows:

HPLC Conditions

HPLC Instrument: Autosampler: Shimadzu SIL-10AXL
Controller: Shimadzu SCL-10A
Pumps: Shimadzu LC-10AT

Mobile Phase: 1 = 0.1% acetic acid
2 = 0.1% acetic acid in ACN

Step	Time	Flow	Grad.	Sol. 1	Sol. 2	Event 1	Event 2
0.0000	0.0000	1000	0.0000	40.0000	60.0000	Open	Open
1.0000	6.0000	1000	0.0000	40.0000	60.0000	Open	Open
2.0000	6.1000	1000	0.0000	10.0000	90.0000	Open	Open
3.0000	9.0000	1000	0.0000	10.0000	90.0000	Open	Open
4.0000	9.1000	1000	0.0000	40.0000	60.0000	Open	Open
5.0000	15.0000	1000	0.0000	40.0000	60.0000	Open	Open

Column:	4.6 x 250-mm Prodigy ODS3, 5 µm ps,
Oven:	Timberline @ 45 θC
Flow Rate:	1000 µL/min, split with nominal 200 µL/min to source
Injection Volume:	10 µL
Retention Time:	SL-160 = approximately 5.50 min TPSA = approximately 3.06 min DTPU = approximately 4.10 min DTPP = approximately 5.94 min ADMP = approximately 3.11 min
Run Time:	15 minutes

Mass Spectrometer Conditions

LC/MS Instrument:	PE-Sciex API300/365 Tandem Mass Spectrometer
API Source:	Turbo Ion Spray (300 θC)
MS Mode:	Tandem (MS/MS) Positive
MS Parameters:	Orifice 45/30/35/35/25 Ring 260/220/280/260/180 Collision Energy 30/27/18/30/22
Mass Calibration:	Positive Mode: Based on PPG masses; 59, 175.133, 616.464, 906.673, 1254.925, 1545.134, 2010.469, 2242.637
Masses Monitored:	408 → 182 SL-160 227 → 146 TPSA 344 → 301 DTPU 301 → 281 DTPP 156 → 100 ADMP
Dwell Time:	1000 ms SL-160 1000 ms TPSA 1000 ms DTPU 1000 ms DTPP 1000 ms ADMP
Software:	PE/Sciex MassChrom 1.0

B. Quantitation and Example Calculation

Standards were injected at the beginning and after approximately every two to three samples throughout the run to generate linear regression calibration curves for SL-160, TPSA, DTPU, DTPP, or ADMP. Quantitation of the amount of SL-160, TPSA, DTPU, DTPP, or ADMP found in an unknown sample was accomplished by inserting the analyte peak area into the appropriate linear regression equation. From the nanograms found, the residue ppm was computed. Since no significant control contribution was detected, no correction of the recoveries for a control contribution was needed. The residue ppm was determined from the following equations:

1. Calculation of ng Found

$$\text{Amount (ng found) in injected sample} = \frac{\text{peak area response - y-intercept}}{\text{slope}}$$

2. Calculation of mg-Equivalent Injected

$$\text{Milligram equivalents injected} = \frac{\text{g. sample x mL aliq x µL injected}}{\text{mL extract. vol x mL final vol x dil factor}} \times \frac{\text{mL}}{1000\ \mu\text{L}} \times \frac{1000\ \text{milligram}}{1\ \text{gram}}$$

3. Calculation of ppm Found

$$\text{ppm found} = \frac{\text{ng found}}{\text{mg-equivalent injected}}$$

4. Calculation of Percent Recovery in Fortification Samples

$$\%\ \text{Recovery} = \frac{\text{ppm found}}{\text{ppm added}} \times 100$$

5. Example Calculation for a Procedural Recovery Sample

DTPU recovery calculated for soil sample ES2364-S5, fortified with 0.025 ppm (25 ppb) DTPU, Set # 1-01-MV, LC Run #80341, See figure 6C.

Sample weight	= 50 g
Extract volume	= 200 mL
Aliquot volume	= 200 mL
Injection volume	= 10 µL
Final volume	= 15mL
Dilution volume	= 10
Peak area	= 11683 counts
y-intercept	= 71.49967
slope	= 146434.7497
Fortification level (ppm)	= 0.025 ppm

ng found = [11683 counts – 71.49967]/146434.7497 = 0.0793 ng

$$\text{mg equiv. injected} = \frac{50\text{ g} \times 200\text{ mL} \times 10\ \mu\text{L} \times 1000\text{ mg/g}}{200\text{ mL} \times 15\text{ mL} \times 10 \times 1000\ \mu\text{l/mL}} = 3.333\text{ mg-equiv.}$$

$$\text{ppm found} = \frac{0.0793\text{ ng}}{3.333\text{ mg}} = 0.023788\text{ ppm}$$

$$\%\text{ Recovery} = \frac{0.023788\text{ ppm}}{0.025\text{ ppm}} \times 100 = 95\%$$

C. Conduct of Trials

The trial consisted of two subsets. Subset 1 included one reagent blank, one unfortified control sample, three fortifications at the Limit of Quantitation (LOQ, 2.5 ppb) and two fortifications at 25 ppb with each of the five analytes. Subset 2 included one unfortified control sample, two fortifications at the Limit of Quantitation (LOQ, 2.5 ppb) and three fortifications at 25 ppb with each of the five analytes.