PART A – BARE SOIL

Methodology was validated (20 and 21 July 2006) to quantify the amount of furfural, furfuryl alcohol, and 2-furoic acid present in bare soil. Recovery samples were extracted twice with 50:50 methanol:reagent water and then centrifuged. Recovery samples were analyzed by automated injection on a high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV).

The bare soil method validation fortification levels for each test substance were as follows:

	Validation Fortification Levels (µg a.i./g)		
Test Substance	**Low**	**Middle**	**High**
furfural	0.500	12.5	150
furfuryl alcohol	0.498	12.5	149
2-furoic acid	0.510	12.8	153

PART B – THATCH/TURF

Methodology was validated (27 July to 4 August 2006) to quantify the amount of furfural, furfuryl alcohol, and 2-furoic acid present in thatch/turf. Furfuryl alcohol and 2-furoic acid were dosed together and furfural was dosed on the thatch/turf separately. Recovery samples were extracted and analyzed following identical procedures to those presented in Part A – Bare Soil method validation.

The thatch/turf method validation fortification levels for each test substance were as follows:

	Validation Fortification Levels (µg a.i./g)		
Test Substance	**Low**	**Middle**	**High**
Furfural	0.500	12.5	150
furfuryl alcohol	0.996	12.5	149
2-furoic acid	1.02	12.8	153

EXPERIMENTAL

Equipment

1.	Instrument:	Hewlett-Packard quaternary solvent pump Series 1100 equipped with a Hewlett-Packard Series 1100 autosampler, degasser, and diode array detector (DAD), and a Hewlett-Packard ChemStation Version A.06.03 for data acquisition.
2.	Balances:	Mettler Toledo AB204, Mettler PJ3000
3.	Centrifuge:	Beckman GPR, Baxter Biofuge 17/169722
4.	Laboratory equipment	gyratory shaker table, syringes, volumetric flasks, volumetric pipets, Pasteur pipets, Nalgene® ultra-centrifuge tubes, autosampler vials, and amber glass bottles with Teflon®-lined caps.

Reagents

1.	Acetonitrile:	Burdick & Jackson, reagent grade
2.	Ethanol:	EMD, reagent grade
3.	Methanol	Burdick and Jackson, reagent grade
4.	Phosphoric acid:	EMD, reagent grade
5.	Reagent grade water:	Prepared from a Sybron/Barnstead NANOpure® water purification system (meets ASTM Type II requirements)

Test Substances

The test substance, furfural technical from Illovo Sugar Ltd., was received on 2 June 2005 via Product Safety Labs, Dayton, New Jersey. The following information was provided by Illovo Sugar Ltd:

Name:	furfural technical
Lot No.:	0503-2795
CAS No.:	98-01-1
Purity:	99.66%
Recertification Date:	14 September 2007 (See Springborn Certificate of Analysis in Appendix 2)

Upon receipt at Springborn Smithers, the test substance (SSL No. 112-78) was stored at room temperature in the original container in a dark ventilated cabinet.

The test substance, 2-furoic acid, was received on 2 December 2003 from Aldrich Chemical, Allentown, Pennsylvania. The following information was provided by the Supplier:

Name:	2-furoic acid
Lot No.:	07614CO
CAS No.:	88-14-2
Purity:	99.9%
Recertification Date:	21 November 2006

Upon receipt at Springborn Smithers, the test substance (SSL No. 101-06) was stored at room temperature in the original container in a dark ventilated cabinet. Concentrations were adjusted for the purity of the test substance and are presented as active ingredient (a.i.).

The test substance, furfuryl alcohol, was received on 2 January 2004 from Sigma-Aldrich, Allentown, Pennsylvania. The following information was provided by the Supplier:

Name:	furfuryl alcohol
Lot No.:	09809DA
CAS No.:	98-00-0
Purity:	98.5% (Certificate of Analysis; Appendix 2)
Recertification Date:	Not listed

Upon receipt at Springborn Smithers, the test substance (SSL No. 101-74) was stored at room temperature in the original container in a dark ventilated cabinet. Concentrations were adjusted for the purity of the test substance and are presented as active ingredient (a.i.).

PROCEDURES

Preparation of Stock Solutions

Primary stocks were prepared for each test substance by placing 0.5022 g, 0.4980 g, and 0.5098 g (as active ingredient) of furfural, furfuryl alcohol and 2-furoic acid, respectively, in separate 50.0-mL volumetric flasks and bringing to volume with the appropriate solvent. Ethanol was used for the 2-furoic acid stock, and acetonitrile was

used for the furfuryl alcohol and furfural stocks. The primary stocks had the following concentrations as each test substance:

Test Substance	Primary Stock Concentration (mg a.i./mL)
furfural	10.0
2-furoic acid	10.2
furfuryl alcohol	9.96

All three stocks were combined as needed to formulate three secondary stock solutions which contained all three test substances. The secondary stocks had the following concentrations as each test substance:

Test Substance	Secondary Stock Concentrations (mg a.i./L)
furfural	10.0, 100 and 1000
2-furoic acid	10.2, 102 and 1020
furfuryl alcohol	9.96, 99.6 and 996

All stock solutions were stored refrigerated in glass amber bottles fitted with Teflon®-lined caps.

Preparation of Calibration Standards

Calibration standards were prepared in 50:50 methanol: reagent water with the secondary stock solutions to yield the following calibration standard concentrations:

Test Substance	calibration standard concentrations (mg a.i./L)
furfural	0.0250, 0.0500, 0.100, 0.250, 0.350, 0.500
furfuryl alcohol	0.0249, 0.0498, 0.0996, 0.249, 0.349, 0.498
2-furoic acid	0.0255, 0.0510,0.102, 0.255, 0.357, 0.510

Sample Fortification and Preparation

A set of recovery samples was prepared by fortifying bare soil (approximately 5.00 g as dry weight) with the appropriate secondary stock solution at concentrations of approximately 0.500, 12.5 and 150 µg a.i./g. Each concentration level was produced in triplicate. In addition, three recovery samples were left unfortified to serve as controls.

Sample Extraction

The recovery samples (5.00 g as dry weight) were extracted with 50:50 methanol:reagent water (20 mL). The solutions were placed on a shaker table set at 150 rpm for one hour. After one hour, the samples were centrifuged at 3000 rpm for 15 minutes and the

supernatant was decanted. The extraction procedure was repeated with an additional 20 mL of 50:50 methanol:reagent water and placed on a shaker table at 150 rpm overnight. The extracts were combined in a volumetric flask and brought to a final volume of 50 mL with 50:50 methanol:reagent water. Mid- and high-concentration recovery samples were diluted into the calibration range using 50:50 methanol:reagent water. The recovery samples were centrifuged at 13,000 rpm for five minutes and an aliquot was transferred to an amber autosampler vial and analyzed by HPLC/UV. In addition, the extracts were left at room temperature overnight and were reanalyzed the next day to confirm 24-hour room temperature stability.

ANALYSIS

Instrumental Conditions

The high performance liquid chromatographic (HPLC) analysis was conducted utilizing the following instrumental conditions:

Column:	Phenomenex Synergi Hydro-RP, 80Å, 4.0 µm, 250 mm x 4.6 mm
Mobile Phase (A):	0.05% phosphoric acid in reagent water
Mobile Phase (B):	100% acetonitrile

Gradient:

Time (min.)	Solvent A	Solvent B
0.00	100.0	0.0
5.00	100.0	0.0
20.0	60.0	40.0
21.0	0.0	100.0
24.0	0.0	100.0
25.0	100.0	0.0

Flow Rate:	1.0 mL/minute
Injection Volume:	50 µL
Wavelength:	280 nm (furfural) 250 nm (2-furoic acid) 215 nm (furfuryl alcohol)
Column Temperature:	ambient
Run Time:	25 minutes
Equilibration Delay:	6.0 minutes
Retention Time:	approximately 16.5 minutes (furfural) approximately 14.9 minutes (furfuryl alcohol) approximately 16.0 minutes (2-furoic acid)

Preparation of Standard Curve

Two sets of calibration standards were analyzed with each sample set; one set prior to analysis of the recovery samples, and the second set immediately following the analysis of the recovery samples. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

CALCULATIONS

A calibration curve was constructed by plotting the analyte concentration (mg a.i./L) of the calibration standards against the peak area of the analyte in the calibration standards. The equation of the line (equation 1) was algebraically manipulated to give equation 2. The concentration of test substance in each recovery sample was calculated using the slope and intercept from the linear regression analysis, the detector response, and the dilution factor of the recovery sample. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

$$(1)\quad y = mx + b$$

$$(2)\quad DC(x) = \frac{(y - b)}{m}$$

$$(3)\quad A = DC \times DF$$

where:

x	=	analyte concentration
y	=	detector response (peak area) from the chromatogram
b	=	y-intercept from the regression analysis
m	=	slope from the regression analysis
DC (x)	=	detected concentration (µg a.i./mL) in the sample
DF	=	dilution factor (final volume of the sample divided by the original sample mass)
A	=	analytical result (µg a.i./g), concentration in the original sample

The limit of quantitation (LOQ) was calculated using the following equations:

$$(4)\ Area_{MIN} = (0.5 \times A_{LS})$$

$$(5)\ LOQ_{INST} = \frac{((0.5 \times A_{LS}) - b)}{m}$$

$$(6)\ LOQ = LOQ_{INST} \times DF_{CNTL}$$

where:

$Area_{MIN}$	=	one half of the mean detector response (peak area) of the low concentration calibration standard (two injections)
A_{LS}	=	mean detector response (peak area) of the low concentration calibration standard (two injections)
b	=	y-intercept of the linear regression
m	=	slope of the linear regression
LOQ_{INST}	=	limit of quantitation at the instrument
DF_{CNTL}	=	dilution factor of the control samples (smallest dilution factor used)
LOQ	=	limit of quantitation reported for the analysis

The following equation was used to calculate percent recoveries:

$$P = A/NC \times 100$$

where:

P	=	Percent recovery of the sample
A	=	Analytical result concentration
NC	=	Nominal fortification concentration

All samples were retained on the instrument overnight for 24 hours at room temperature to verify stability of the test substances in the solvent extract. Following 24 hours, samples were reinjected onto the HPLC system. All of the sample results remained generally consistent with the original method validation results, and therefore confirm 24-hour stability of the test substances in the solvent extract.