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DETERMINATION OF RESIDUES OF S-3153, S-3153 ACID, S-3153-1-OH, AND S-3153-1-OCH_3 IN SOIL

Method: RM-36-2 Date: October 26, 1999

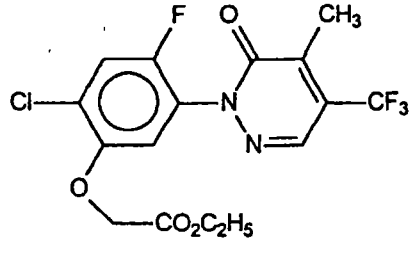
I. INTRODUCTION

This method describes the determination of S-3153 {ethyl [2-chloro-4-fluoro-5-(5-methyl-6-oxo-4-trifluoromethyl-1,6-dihydropyridazin-1-yl)phenoxy]acetate} and its degradates, S-3153 Acid {[2-chloro-4-fluoro-5-(5-methyl-6-oxo-4-trifluoromethyl-1,6-dihydropyridazin-1-yl)phenoxy] acetic acid}, S-3153-1-OH {2-(4-chloro-2-fluoro-5-hydroxy)phenyl-4-methyl-5- trifluoromethyl-2,3-dihydropyridazin-3-one}, and S-3153-1-OCH_3 {2-(4-chloro-2-fluoro-5-methoxy)phenyl-4-methyl-5-trifluoro-methyl-2,3-dihydropyridazin-3-one}, in soil.

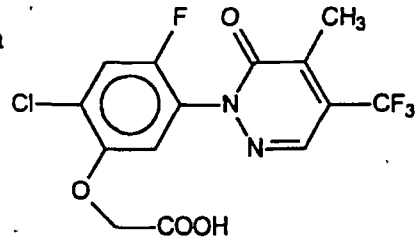
The method involves extracting the residues into 4/1 acetone/0.01 *N* HCl (v/v); rotary evaporating the acetone to obtain an aqueous residue; adding Hastings-Sendroy buffer to set the pH to 6; partitioning the S-3153, S-3153-1-OH, and S-3153-1-OCH_3 residues into 9/1 hexane/acetone (v/v); resetting the pH to 2 with HCl; and then partitioning the S-3153 Acid residues into methylene chloride. The extract containing the S-3153, S-3153-1-OH, and S-3153-1-OCH_3 residues is then rotary evaporated, the residues are dissolved in 1.0 mL of ethyl acetate, and the final extract is analyzed by GC/MSD. For the S-3153 Acid residues, the methylene chloride is removed by rotary evaporation, the residues are dissolved in ethanol, the ethanol mixture is acidified with concentrated HCl, and the acidic mixture is refluxed for 30 minutes. After cooling the reaction mixture, water is added and the S-3153 (formed by the ethylation of the S-3153 Acid) is extracted with methylene chloride. The methylene chloride extract is washed with buffer to remove traces of acid, the methylene chloride is then removed by rotary evaporation, and the residues are dissolved in 1.0 mL of ethyl acetate. Analysis for S-3153 Acid (as S-3153) is performed by GC/MSD.

II. ANALYTICAL STANDARDS

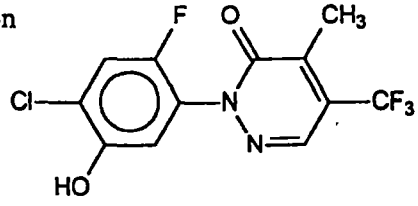
S-3153 (or V-3153) reference standard
- Valent U.S.A. Corporation



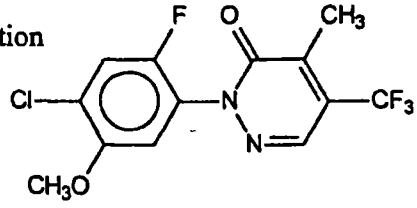
S-3153 Acid reference standard - Valent U.S.A. Corporation



S-3153-1-OH reference standard - Valent U.S.A. Corporation



S-3153-1-OCH_3 reference standard - Valent U.S.A. Corporation



S-3153 standard, 0.25 mg/mL Stock solution.

Weigh 0.025 grams (to ensure a 0.25 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into 100 mL volumetric flask. Dilute to volume with acetone, and store refrigerated.

S-3153 Acid standard, 0.25 mg/mL Stock solution.

Weigh 0.025 grams (to ensure a 0.25 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into 100 mL volumetric flask. Dilute to volume with acetone, and store refrigerated.

S-3153-1-OH standard, 0.25 mg/mL Stock solution.

Weigh 0.025 grams (to ensure a 0.25 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into 100 mL volumetric flask. Dilute to volume with acetone, and store refrigerated.

S-3153-1-OCH_3 standard, 0.25 mg/mL Stock solution.

Weigh 0.025 grams (to ensure a 0.25 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into 100 mL volumetric flask. Dilute to volume with acetone, and store refrigerated.

Mixed Standard (S-3153, S-3153-1-OH, & S-3153-1-OCH_3), 50 µg/mL solution (in acetone).
Pipet 10.0 mL of the each of the three Stock solutions into a 50 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

Mixed Standard (S-3153, S-3153-1-OH, & S-3153-1-OCH_3), 2.0 µg/mL solution (in acetone).
Pipet 4.0 mL of the Mixed Standard 50 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

Mixed Standard (S-3153, S-3153-1-OH, & S-3153-1-OCH_3), 0.5 µg/mL solution (in acetone).
Pipet 25.0 mL of the Mixed Standard 2.0 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

S-3153 Acid Standard, 50 µg/mL solution (in acetone).
Pipet 10.0 mL of the Stock solution into a 50 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

S-3153 Acid Standard, 2.0 µg/mL solution (in acetone).
Pipet 4.0 mL of the S-3153 Acid Standard 50 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

S-3153 Acid Standard, 0.5 µg/mL solution (in acetone).
Pipet 25.0 mL of the S-3153 Acid Standard 2.0 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

Mixed Standard (S-3153, S-3153-1-OH, & S-3153-1-OCH_3), 1.0 µg/mL in ethyl acetate.
Pipet 2.0 mL of the Mixed Standard 50 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with ethyl acetate. Store refrigerated.

Mixed Standard (S-3153, S-3153-1-OH, & S-3153-1-OCH_3), 0.5 µg/mL in ethyl acetate.
Pipet 1.0 mL of the Mixed Standard 50 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with ethyl acetate. Store refrigerated.

Mixed Standard (S-3153, S-3153-1-OH, & S-3153-1-OCH_3), 0.1 µg/mL in ethyl acetate.
Pipet 5.0 mL of the Mixed Standard 2.0 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with ethyl acetate. Store refrigerated.

Mixed Standard (S-3153, S-3153-1-OH, & S-3153-1-OCH_3), 0.04 µg/mL in ethyl acetate.
Pipet 2.0 mL of the Mixed Standard 2.0 µg/mL solution (in acetone) into a 100 mL volumetric flask and dilute to volume with ethyl acetate. Store refrigerated.

Note: Similar serial dilutions may also be performed to generate the appropriate standards.

III. REAGENTS

Acetone - Pesticide quality

Disodium Hydrogen Phosphate·$7H_2O$ [$Na_2HPO_4 \cdot 7H_2O$] - Reagent grade

Ethyl Acetate - Pesticide quality

Hexane - Pesticide quality

Hydrochloric Acid, Concentrated [12 *N*]- Reagent Grade

Methylene Chloride - Pesticide quality

Potassium Phosphate, Monobasic [KH_2PO_4] - Reagent grade

Sodium Sulfate - AR grade, granular (acetone-washed and air dried)

Water – Deionized

IV. REAGENT SOLUTIONS

Acetone/0.01 *N* HCl, 4/1 (v/v).

Combine 4 parts acetone with 1 part 0.01 *N* HCl. For example, add 800 mL of acetone and 200 mL of 0.01 *N* HCl sequentially to a reagent bottle. Store at room temperature.

Hastings-Sendroy Buffer.

Combine 611 mL of 1/15 *M* disodium hydrogen phosphate with 389 mL of 1/15 *M* potassium phosphate in an erlenmeyer flask (or reagent bottle). Mix well. [The amounts used may be scaled, as appropriate.] Store at room temperature.

Hexane/Acetone, 9/1 (v/v).

Combine 9 parts hexane with 1 part acetone. For example, add 900 mL of hexane and 100 mL of acetone sequentially to a reagent bottle. Store at room temperature.

Hydrochloric acid, 1 *N*

Dilute 80 mL of 12 *N* hydrochloric acid to 960 mL with deionized water. For example - add approximately 500 mL deionized water into a large beaker or flask, add the 12 *N* acid, transfer the dilute solution into a 1000 mL graduated cylinder, and dilute to volume with deionized water. [Similar ratios of acid to water may also be used.] Store at room temperature. *Use caution when handling 12 N HCl as it is corrosive - avoid breathing fumes, and avoid contact with skin or clothing.*

Hydrochloric acid, 0.01 *N*

Add 10 mL of 1 *N* HCl to 990 mL of deionized water. Or, add 0.8 mL of 12 *N* hydrochloric acid to 960 mL of deionized water. [Similar ratios of acid to water may also be used.] Store at room temperature.

Potassium Phosphate, 1/15 *M*

Prepare a 1/15 *M* aqueous solution by adding 9.07 grams KH_2PO_4 into 1 L of deionized water in an erlenmeyer flask (or reagent bottle). Cover (or stopper) and stir (or shake) the mixture to dissolve the KH_2PO_4. [The amounts used may be scaled, as appropriate.] Store at room temperature.

Disodium Hydrogen Phosphate, 1/15 *M*

Prepare a 1/15 *M* aqueous solution by adding 17.9 grams $Na_2HPO_4 \cdot 7H_2O$ into 1 L of deionized water in an erlenmeyer flask (or reagent bottle). Cover (or stopper) and stir (or shake) the mixture to dissolve the $Na_2HPO_4 \cdot 7H_2O$. [The amounts used may be scaled, as appropriate.] Store at room temperature.

V. EOUIPMENT

Balances, Analytical and Top Loading

Bηchner Funnels - 9 cm

Filter Flasks, Vacuum - 500 mL

Filter Funnels (approximately 100 mm diameter)

Filters, Glass Fiber - Whatman GF/A, 9 cm

Graduated Cylinders (1000, 250, 100, 50, 25 mL)

Glass Wool, Pyrex

Heated Water Bath (temperature <40°C)

Heating Mantles

Linear Shaker - Erbach (or equivalent)

Mason Jars - pint (or equivalent)

Pasteur Pipettes, Disposable - 5: and 9 inch

Pipettors, Automatic - capable of accurately dispensing volumes of 0.1 to 2.0 mL

Pipettes, Volumetric - 25.0, 10.0, and 4.0 mL

Round-bottom Flasks (500, 250, 100, and 50 mL)

Reflux condensers

Refrigerator

Rotary Vacuum Evaporators

Separatory Funnels (250 and 125 mL)

VI. INSTRUMENTATION

GAS CHROMATOGRAPH (GC)

Hewlett-Packard Model 5890 GC equipped with a Hewlett-Packard Model 5971 mass selective detector (GC/MSD), autosampler, and integrator (or equivalent). The conditions shown are suggested for this analysis, and these may be changed as appropriate (*see Note 1 for additional parameters*).

Column:	DB - 1 (J & W Scientific, Inc.), 30 m x 0.25 mm I.D. , 0.25 µm film thickness (or equivalent).	
Carrier gas :	Helium, 0.8 mL/min.	
Injector:	1.0 µL, Splitless, 0.8 min. Purge valve delay	
Temperatures:	Injector -	240°C
	Detector -	280°C
	Column Oven -	75°C Initial (Hold 1.0 min.)
		30°C/min Ramp to 225°C (Hold 1.5 min.)
		25°C/min Ramp to 280°C (Hold 1.0 min.)
		35°C/min Ramp to 310°C (Hold 4.0 min.)
Retention Times (approximate):		7.9 minutes (S-3153-1-OH)
		8.1 minutes (S-3153-1-OCH_3)
		9.7 minutes (S-3153)

Note: A chromatogram of the Mixed Standard is shown in Figure 1.

VII. ANALYTICAL PROCEDURES

1. Sample Setup

Weight 20 g of the soil into a pint Mason® jar. At this point, if required by the testing facility, a control sample to be used for method recoveries may be fortified with S-3153, S-3153 Acid, S-3153-1-OH, and S-3153-1-OCH_3 (*see Note 2*).

2. Extraction with Acetone/0.01 *N* HCl

Add 100 mL of 4/1 acetone/0.01 *N* HCl into the pint jar. Place the cover on the jar and then shake on the linear shaker for 30 minutes. Filter the mixture through a Whatman® GF/A glass filter in a Büchner funnel (under suction) into a 500 mL filter flask. Transfer the moist solids (and the glass fiber filter) back into the jar, and re-extract the solids by adding a second 100 mL portion of 100 mL of 4/1 acetone/0.01 *N* HCl and shaking for 20 minutes. Filter as before, rinse the jar with 50 mL of 4/1 acetone/0.01 *N* HCl, and pass the rinse through the solids on the glass fiber filter - combining the rinse with the extracts.

3. Partition with 9/1 Hexane/Acetone

Transfer the filtrate into a 500 mL round-bottom flask, and rotary evaporate the mixture to remove the acetone (temperature <40°C).

Transfer the aqueous residue (about 45 mL) into a 250 mL separatory funnel, and add 2.0 mL of Hastings-Sendroy buffer to the aqueous residue. Check that the pH is approximately 6. Rinse the round-bottom flask with 100 mL of 9/1 hexane/acetone, and transfer this rinse into the separatory funnel. Partition the S-3153, S-3153-1-OH, and S-3153-1-OCH_3 residues into the hexane/acetone by shaking vigorously for 1 minute. Allow the phases to separate, and then drain the aqueous layer back into the round-bottom flask. Drain the hexane/acetone layer through approximately 80 g of sodium sulfate (suspended on a glass wool plug in a glass filter; and freshly washed with 9/1 hexane/acetone) into a 500 mL round-bottom flask.

Transfer the aqueous residue from the round-bottom flask back into the separatory funnel, rinse this round-bottom flask with 100 mL of 9/1 hexane/acetone, and then transfer the rinse into the separatory funnel. Partition the sample by shaking for 1 minute. As before, allow the phases to separate, drain the aqueous layer back into the initial 500 mL round-bottom flask, and then drain the hexane/acetone layer through the sodium sulfate - combining this extract with the first. Rinse the sodium sulfate with 10-20 mL of 9/1 hexane/acetone, and collect the rinsate with the combined extracts. ***Reserve this combined hexane/acetone extract for concentration - Step 6.***

4. Partition with Methylene Chloride (S-3153 Acid)

Add 2.0 mL of 1 *N* HCl to the aqueous residues. Check that the pH is approximately 2. Transfer the solution back into the 250 mL separatory funnel, rinse the round-bottom flask with 50 mL of methylene chloride, and add the methylene chloride rinse to the separatory funnel. Shake the sample vigorously for 1 minute, with venting. After the phases separate, drain the methylene chloride layer (the lower layer) into a clean 500 mL round-bottom flask.

Rinse the round-bottom that had held the aqueous residues with a second 50 mL portion of methylene chloride, transfer this rinse into the separatory funnel, and shake again for 1 minute. Allow the phases to separate, and drain the methylene chloride layer into the 500 mL round-bottom flask - combining the methylene chloride extracts. Repeat the extraction with a third 50 mL portion of methylene chloride - combining the extracts as before.

5. Ethylation (S-3153 Acid)

Rotary evaporate (temperature <40°C) the methylene chloride to reduce the volume to 5-10 mL. Transfer the residues to a 50 mL round-bottom flask, using two 10 mL methylene chloride rinses of the 500 mL round-bottom flask. Continue rotary evaporation to remove the methylene chloride (temperature <40°C), just to dryness.

Add 10 mL of ethanol to the flask, and sonicate briefly to dissolve the residues. Add 0.5 mL of concentrated HCl to the sample, add a Teflon boiling chip, attach the round-bottom flask to a reflux condenser, and place the flask into a heating mantle. Reflux the mixture for 30 minutes, and then allow the sample to cool (20-30 minutes).

Add 25-30 mL of deionized water to the sample, transfer the residues into a 125 mL (or 250 mL) separatory funnel, rinse the 50 mL round-bottom flask with 25 mL of methylene chloride, and add the rinsate to the separatory funnel. Shake the sample vigorously for 1 minute (with venting) to partition the S-3153 (ethylated S-3153 Acid) into the methylene chloride layer. Allow the phases to separate, and then drain the methylene chloride layer into a clean 100 mL round-bottom flask (or similar container). Rinse the 50 mL round-bottom flask with a second 25 mL portion of methylene chloride, transfer the rinse into the separatory funnel, and repeat the extraction. Drain the methylene chloride layer into the flask containing the initial methylene chloride extract. Discard the aqueous layer, and transfer the methylene chloride extract back into the separatory funnel (rinsing the flask with 5 mL of methylene chloride and adding the rinse to the separatory funnel). Add 5 mL of Hastings-Sendroy buffer to the separatory funnel, and shake the sample vigorously for 30 seconds (with venting). Allow the phases to separate, drain the methylene chloride layer through approximately 20-25 g of sodium sulfate (suspended on a glass wool plug in a glass filter; and freshly washed with methylene chloride) into a 250

mL round-bottom flask, and then rinse the sodium sulfate with 10-15 mL of methylene chloride - combining the methylene chloride extracts and rinse.

Rotary evaporate the methylene chloride (using a heated water bath, temperature <40°C) to reduce the volume to 10-20 mL. Transfer the residues to a 100 mL round-bottom flask, using two sequential 10 mL ethyl acetate rinses. Continue rotary evaporation, just to dryness. Add 1.0 mL of ethyl acetate, stopper and sonicate briefly to dissolve the residues, and then transfer the final extract into an autosampler vial (or vials) or a screw top vial for storage under refrigeration. Samples in autosampler vials may also be analyzed directly.

6. Concentration for S-3153, S-3153-1-OH, and S-3153-1-OCH$_3$

For the reserved extract from Step 2, rotary evaporate the solvent (temperature <40°C) to reduce the volume to 10-20 mL. Transfer the residues to a 100 mL round-bottom flask, using two sequential 10 mL ethyl acetate rinses. Continue rotary evaporation, just to dryness. Add 1.0 mL of ethyl acetate to the flask, and then stopper and sonicate briefly to dissolve the residues. Transfer this extract into an autosampler vial (or vials) or a screw top vial for storage under refrigeration. Samples in autosampler vials may also be analyzed directly.

7. GC Measurement

Condition the instrument with at least three injections of a sample extract (*see Note 3*). Analyze a range of at least four standard concentrations using Mixed Standards (in ethyl acetate) *within the analytical sequence* to establish the linear response of the GC/MSD, including a 0.04 µg/mL standard. [A typical standard sequence would be 0.04, 0.1, 0.5, and 1.0 µg/mL, with an injection volume of 1.0 µL.] To verify the linear response, calculate the response factor for each of the four standards [by dividing the peak height (or area) of the standard by its concentration], determine the standard deviation of the four response factors, and then divide this value by the average response factor. The result (the coefficient of variation) must be 15% or less for the instrument response to be considered linear for that range of standards.

It is recommended that the linearity standards be interspersed within the analytical run, as this allows the injection port and column to be conditioned with sample matrix from the preceding injection. A sample sequence is then constructed with the following order: a reference standard (0.5 µg/mL), a set of 2 to 4 sample extracts, a reference standard or linearity standard, another set of 2 to 4 sample extracts, ..., and a reference standard. *The sequence must begin and end with reference standards*. The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak response for any of the analytes in a sample is greater than the peak response of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed.

The sample extract must be diluted (with acetone) such that the peaks obtained are within the documented linear response range of the GC/MSD.

7. Calculations

The amount of S-3153, S-3153-1-OH, and S-3153-1-OCH_3 in each sample is calculated as follows:

$$\text{Sample concentration, ppm } (\mu g/g) = \frac{A \times B \times C \times D}{E \times F}$$

where:
A = Sample peak height (or area)
B = Continuing calibration standard concentration (0.5 µg/mL)
C = Final extract volume (1.0 mL)
D = Dilution factor, if any
E = Average calibration standard peak height (or area)
F = Initial sample weight (20 g)

The amount of S-3153 Acid (after conversion to S-3153) is calculated as follows:

$$\text{Sample concentration, ppm } (\mu g/g) = \frac{A \times B \times C \times D \times E}{F \times G}$$

where:
A = Sample peak height (or area)
B = Continuing calibration standard concentration (0.5 µg/mL)
C = Final extract volume (1.0 mL)
D = Dilution factor, if any
E = Conversion Factor ($MW_{S\text{-}3153\ Acid}/MW_{S\text{-}3153} = 0.93$)
F = Average calibration standard peak height (or area)
G = Initial sample weight (20 g)

VIII. LIMIT OF DETECTION

The limit of detection (LOD) of this method is 0.002 ppm (µg/g) for each analyte; and the validated limit of quantitation (LOQ) is 0.005 ppm (µg/g).

X. NOTES

1. The mass selective detector parameters shown below are suggested for this analysis - similar conditions may be used as appropriate:

Acquisition Mode:	Selective Ion Monitoring (SIM)	
Solvent Delay:	7 minutes	
MSD Off:	11 minutes	
Quantitation Ions:	S-3153	345 + 335
	S-3153-1-OH	322 (parent ion) + 303
	S-3153-1-OCH_3	336 (parent ion) + 317

2. Valent Standard Operating Procedure (SOP) VR-002 specifies that fortified control samples are to be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if an untreated control (UTC) sample is not available, this method requirement may be waived.

   The level of fortification is generally 0.005 ppm (the limit of quantitation of the method) and/or 0.025 ppm. Add 0.20 mL of the 0.5 µg/mL Mixed Standard solution (in acetone) and 0.20 mL of the 0.5 µg/mL S-3153 Acid Standard solution (in acetone) to obtain a 0.005 ppm fortification level, and/or add 0.25 mL of the 2.0 µg/mL Mixed Standard solution (in acetone) and 0.25 mL of the 2.0 µg/mL S-3153 Acid Standard solution (in acetone) to obtain a 0.025 ppm fortification level in a 20 g sample. Method recoveries must be 70 to 120% to be acceptable (except for S-3153-1-OH, with acceptable recoveries from 80 to 130%), unless approved by the chemist responsible for the analysis.

3. The analysis of extracts and standards by GC/MSD is noticeably influenced by the condition of the quartz liner, the fused-silica wool in the liner, and the gold-plated seal at the base of the injection port. Care should be taken to ensure that the liner is

deactivated (e.g.- by treatment with Sylon CT from Supelco) and that a minimum amount of fused-silica wool is inserted about half to two-thirds of the distance from the top of the liner. Maintenance relating to the injection port is very important for the analysis of both S-3153-1-OH and S-3153. If system maintenance is neglected, apparent enhancement of analytes in samples (relative to standards) will be observed in the order S-3153-1-OH > S-3153 > S-3153-1-OCH_3. *Conditioning of the injection port liner prior to the analysis of a sample sequence is essential to obtain reproducible data.*

XI. SIGNATURES

Written by Charles Green Date 11/2/99
Charles Green

Reviewed by Date 11/4/99
Glenn Fujie, Laboratory Manager

Reviewed by Date 10/28/99
QAU