1. INTRODUCTION

Background and Objective:

The objective of this study was to develop and to validate an analytical method for the determination of S-1563 in soil. The target limit of quantification (LOQ) is 0.01 mg/kg, expressed as S-1563.

Data Requirements and Guidelines:

U.S.EPA guideline, OPPTS. 860.1340, additional guidance on TNsG on Data Requirements, Part A, Chapter 2, Point 4 and Part B, Chapter 2, Point 4 and EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4 11/07/00 and SANCO/825/00 rev. 7 17/03/044).

Method Principles and Method Validation:

Extraction of residues in 10 g soil with acetone, followed by solid-phase extraction with silica gel (SPE clean up) and subsequent elution with 20 mL of n-hexane/ethyl acetate (5/1; v/v). The final volume was adjusted to 1.0 mL using ethyl acetate. Finally the analytes were determined with GC/MS/MS in positive electron impact ionization mode monitoring two parent-daughter ion transitions for each isomer, S-1563RTZ and S-1563RTE separately, for quantitation and quantitative confirmation. The concentration of S-1563 was calculated as sum of the concentrations of both isomers.

For method validation, specimens were fortified (5 replicates per fortification level) at 0.01 mg/kg (LOQ) and at 0.10 mg/kg (10xLOQ). Additional specimens were kept untreated as blank controls.

2. EXPERIMENTAL

2.1 Test System

The European standard soil LUFA 5M (sandy loam; USDA; sieved at PTRL Europe prior to use) was used. Water content of 1 % (determined at the beginning of the study) was neglected in this study.

Details on the soil characteristics as provided with the soil are given in APPENDIX 2. The soil was collected in February-2008 at LUFA Speyer and was stored dry at PTRL Europe.

2.2 Analytical Test and Reference Items

The following standards provided by the Sponsor (see Appendix 1) were used as test / reference items:

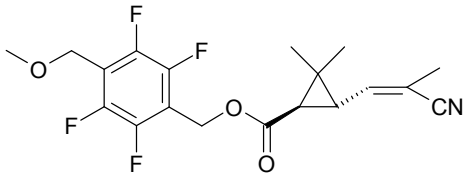
- S-1563RTZ (1R-trans-Z-isomer),

  Chemical Name: 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (*Z*)-(1*R*,3*R*)- 3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
- S-1563RTE (1R-trans-E-isomer),

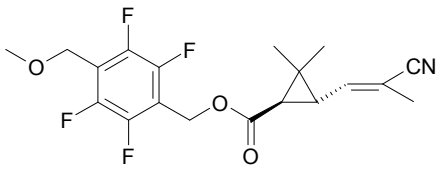
  Chemical Name: 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (E)-(1R,3R)-3-(2-cyanoprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
- S-1563 (Mixture of Isomers, 90 % S-1563 RTZ, 10 % S-1563 RTE)

  Chemical Name: 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl-2-(2-cyanoprop-1-enyl)-3,3-dimethylcyclopropanecarboxylate

S-1563 RTZ



S-1563 RTE



Empirical Formula: $C_{19}H_{19}F_4NO_3$ Molar Mass: 385.36 g/mol

2.3 Analytical Method

2.3.1 Apparatus

2.3.1.1 Laboratory Equipment

Sartorius analytical balance RC 210 D for analytical standards. Sartorius ED 2202S-CW, Scaltec SBC (used for specimens).

SPE station: Baker SPE-12G. Ultrasonic bath Transsonic 700, Schmidbauer. Centrifuge

Rotixa 50 S, Hettich. JKA horizontalshaker HS 250 B. Rotationvacuumconcentrator, Thermo Electron. Reacti-Vap, Thermo/Pierce. Drying Oven Horo, Memmert. Sieve maschine VIBRO, Retsch.

Typical glassware and laboratory equipment.

All the glassware was cleaned in a laboratory dishwasher and air-dried before use.

2.3.1.2 GC/MS System

Thermo TSQ Quantum GC/MS System equipped with TriPlus AS autosampler, Trace Ultra GC gas chromatograph, temperature programmable PTV and split/splitless injector, digital pressure and flow control DPFC.

GC-Column: Varian VF-5 MS (30 m length, 0.25 mm i.d., 0.50 µm film, stationary phase: 5 % phenyl, 95 % methylpolysiloxane) capillary column.

TSQ Quantum triple-quadrupol mass spectrometer, EI ion volume, Xcalibur GC/MS instrument control and data acquisition software.

2.3.2 Solvents and Chemicals

Acetone, ethyl acetate, and n-hexane, all Promochem pesticide or HPLC grade.

2.3.3 Miscellaneous

SPE columns: Mega Bond Elute-SI, silica gel 1g, 6 mL, Agilent Technologies.

2.3.4 Preparation of Standard Solutions

Solution of S-1563:

A stock solution of S-1563 was prepared by accurately weighing 10.49 mg of the analytical standard (taking purity of 95.6 % into consideration) and dissolving it in 10.0 mL of acetone to obtain a concentration of 1.0 mg/mL.

This solution was further diluted volumetrically into acetone to obtain fortification solutions at 4.0 and 0. 40 µg/mL.

For fortification of recoveries at 0.01 mg/kg, the fortification solution with the concentration of 0.40 µg/mL and for recoveries at 0.10 mg/kg, the fortification solution with a concentration of 4.0 µg/mL was used.

Solutions of the isomers S-1563RTZ and S-1563RTE:

A stock solution of S-1563RTZ was prepared by accurately weighing 10.01 mg of the analytical standard (taking purity of 99.7 % into consideration) and dissolving it in 10.0 mL of acetone to obtain a concentration of 1.0 mg/mL.

A stock solution of S-1563RTE was prepared by accurately weighing 10.04 mg of the analytical standard (taking purity of 99.7 % into consideration) and dissolving it in 10.0 mL of acetone to obtain a concentration of 1.0 mg/mL.

Intermediate solutions with both analytes at 20 and 1.0 µg/mL were prepared by volumetric dilution of the stock solutions into ethyl acetate

Calibration solutions with 1.0 to 200 ng/mL of S-1563RTZ and S-1563RTE were prepared by volumetric dilution into ethyl acetate.

Due to a significant matrix effect (see Table 2) matrix-matched standards were prepared for evaluation of the sample extracts.

For preparation of matrix matched standards final extracts of residue-free control specimen (process together with the validation specimens) were used. Aliquots of the final extracts were fortified with S-1563RTZ and S-1563RTE using the intermediate or calibration solutions in solvent resulting in concentrations of 1.0, 10, 50, 100 and 150 ng/mL per analyte.

2.3.5 Stability of Standard Solutions and Extracts

Fortification and standard solutions were stored refrigerated in amber glass bottles when not in use. Stability of standard solutions during the course of the study (about 9 weeks) was demonstrated by consistent GC/MS/MS results. Consistent fortification results demonstrate stability of extracts (at ambient conditions) during the duration of the analysis (approximately 1 day).

2.3.6 Residue Analysis

2.3.6.1 Extraction

1. Place 10 g (W) sample material (dry soil) into a 50-mL centrifuge vial.
2. Fortify the sample, with 0.25 mL of 0.40 µg/mL or of 4.0 µg/mL fortification solution to obtain 0.01 mg/kg or 0.10 mg/kg in dry soil, respectively.
3. Add 20 mL of acetone, cap and shake for 15 min. on a horizontalshaker.
4. Sonicate for 5 min. and centrifuge for 5 min. at 4000 rpm.
5. Filter the supernatant through a filter paper into a 100-mL volumetric cylinder.
6. Repeat extraction steps 3 to 5 two times, combine the filtrates and make up to 60 mL with acetone.
7. Evaporate the filtrate to dryness using a reacti-vap (constant stream of nitrogen) with water bath (temperature below 40 °C).
8. Dissolve the residue in 3 mL hexane using an ultrasonic bath.

2.3.6.2 Solid-phase extraction with silica gel

9. Condition a Mega Bond Elute SI column (1g, 6mL) with 5 mL of ethyl acetate and 10 mL of hexane.

10. Apply the residue from point 8 onto the SPE column, rinse the flask with 3 mL of hexane, followed by 4 mL of hexane. Apply the rinsates onto the column and discard the eluates.
11. Elute the analytes with 20 mL of hexane/ethyl acetate (5/1, v/v) into a 50-mL centrifuge vial.
12. Evaporate the filtrate to dryness using a reacti-vap with water bath (constant stream of nitrogen, temperature below 40°C).
13. Reconstitute the residue in 1.0 mL (V_{End}) of ethyl acetate using an ultrasonic bath.
14. Transfer extract into a suitable autosampler vial and analyse by GC/MS/MS.
15. Dilute 10xLOQ sample extracts by dilution factor DF10 (using final extracts of residue-free control specimens as solvent).

2.4 GC/MS/MS Analysis

Specimen extracts and calibration solutions in matrix were analyzed by capillary gas chromatography with mass spectrometric detection (GC/MS/MS), using the following GC/MS/MS parameters:

Autosampler	Basic injection mode, injection speed 100 µL/sec, injection volume 1 µL.
Injection technique	S/SL injector used: split/splitless injection (splitless time: 2 min). Initial temperature 250 °C
Carrier gas	Helium with constant flow at 1.5 mL/min.
GC capillary column	Varian Factor Four VF-5 MS fused silica capillary column (30 m length, 0.25 mm inner diameter, 0.50 µm film thickness).
Oven temperature program	70 °C, 1 min hold, ramp with 20 °C/min to 240 °C, ramp with 5 °C/min to 260 °C, ramp with 100 °C/min to 320 °C, 3 min hold.
Retention time	S-1563RTZ: ≈ 10.8 min, S-1563RTE: ≈ 11 min.
EI MS Detection	Electron impact (EI) mass spectrometric detection. Emission current: 70 µA Electron energy: -70 eV Source temperature: 260 °C. Selected reaction monitoring (SRM) mode: isolating 177 m/z fragment ion as parent ion and monitoring 127 m/z as daughter ion and isolating 176 m/z fragment ion as parent ion and monitoring 125 m/z as daughter ion for quantification and quantitative confirmation of both analytes.
Calibration	External calibration (regression calculation) with 1/x weighting, employing quantification by peak area of the S-1563RTZ and S-1563RTE peaks in the respective ion chromatograms, using standards in matrix. Linear calibration functions were established by regression calculations, injecting at least 5 calibration levels. 1/x weighting was used for the wide range of the calibration curve.

The quantitative determination was carried out by external standardization using matrix-matched standards. Calibration functions ranging from 1.0 to 150 ng/mL were used to evaluate the extracts (exemplified in Figure 1).

Representative calibration curves and functions for calibrations in solvent are given in Figure 2.

Representative GC/MS/MS ion chromatograms of calibration solutions in matrix and for extracts of fortified and control specimens are presented in Figure 6 to Figure 9.

2.5 Calculations

Results derived from GC/MS/MS and calculations are shown in detail in Table 1.

The following equation was used to calculate the individual residues R in mg/kg:

$R = c_{End} \times [V_{End} / (W \times 1000)\ ng/\mu g] \times DF$

$= c_{End} \times \text{Multiplier M} \times DF$

R: Analyte residue in mg/kg.

c_{End}: Final concentration of S-1563RTZ or S-1563RTE in specimen extract, in ng/mL.

V_{End}: Volume of final extract: 1.0 mL.

W: Sample weight: 10 g.

1000: Divisor used to adjust dimensions.

DF: Dilution Factor, 10 for 10xLOQ sample extracts.

The concentration of the residue for S-1563 was calculated as sum of the concentrations of both isomers obtained by the above equation.

R (S-1563 (mg/kg)) = R (S-1563RTZ (mg/kg)) + R (S-1563RTE (mg/kg))

Recoveries (Rec.) were calculated for the fortified specimens (for S-1563) as follows:

$Rec. = (R / R_{fortified}) \times 100\ \%$

Example for Calculation:

The calculation is exemplified with the soil specimen P2056-164 fortified at 0.010 mg/kg (LOQ). The final extract was examined by GC/MS/MS in run file P2056-413 (Figure 7 and Figure 9) to give a peak area of 398705 counts for the S-1563RTZ isomer in the transition 177 m/z to 127 m/z. Using the respective calibration curve (see Figure 1) a final concentration of 80.6 ng/mL is calculated for the S-1563RTZ isomer (see Table 1).

Thus:

$R = c_{End} \times V_{End} / (W \times 1000)\ ng/\mu g \times DF$

$= c_{End} \times \text{Multiplier M} \times DF$

$= 80.6\ ng/mL \times 1.0\ mL / (10\ g \times 1000)\ ng/\mu g \times 1$

$= 80.6\ ng/mL \times 0.0001\ mL/g \times \mu g/ng \times 1$

$= 0.0081\ \mu g/g \text{ or } mg/kg$

In the same way, a final concentration C_{End} for the S-1563RTE isomer was calculated to be 0.00078 mg/kg.

R (S-1563 (mg/kg)) = R (S-1563RTZ (mg/kg)) + R (S-1563RTE (mg/kg))

= 0.0081 mg/kg + 0.00078 mg/kg

= 0.0088 mg/kg

Rec. = $(R / R_{fortified}) \times 100$ %

= (0.0088 mg/kg / 0.010 mg/kg) x 100 % = 88 %.

Calculations were performed with full precision. Thus discrepancies may arise when recalculated.

The objective of the study was to develop and to validate an analytical method for the determination of S-1563 in soil, with a limit of quantitation (LOQ) of 0.01 mg/kg.

All specimens were analysed by gas chromatography in electron impact ionization mode with mass selective detection (GC/MS/MS). For quantitation and quantitative confirmation two parent-daughter ion transitions for the two isomers in S-1563 were monitored.

3.1 Sensitivity, Calibration, Specificity

The highly specific GC/MS/MS method uses for both isomers two mass transitions 177 m/z → 127 m/z and 176 m/z → 125 m/z for quantitation and quantitative confirmation.

GC/MS/MS using electron impact ionization (EI) and selected reaction monitoring (SRM) mode allows to detect S-1563RTZ and S-1563RTE at concentrations down to 1.0 ng/mL with 1.0-µL injections, therefore providing sufficient sensitivity to determine and to confirm residues of the analytes in the final extracts. Calibration functions obtained from injections of calibration solutions in matrix consisting of at least 5 different concentrations ranging from 1.0 to 150 ng/mL were used to evaluate the specimen extracts, as exemplified in Figure 1. Linear calibration functions with 1/x weighting were calculated and plotted by regression analysis, using the GC/MS Xcalibur software.

Matrix effects were tested by evaluating GC/MS/MS response of standards in solvent with standards in matrix. Significant matrix effects on GC/MS/MS response were observed (see Table 2), thus sample extracts were evaluated with linear calibration functions at ≥ 5 different concentrations based on matrix-matched standards.

In the case that other soil types and/or different GC/MS instrumentation are used, effects of matrix on response have to be examined, and if significant, compensated by the use of matrix matched standards.

As demonstrated by the method validation results, the method allows the determination of S-1563 with a limit of quantification (LOQ) of 0.01 mg/kg. The limit of detection (LOD) of the method was set to 2 % of LOQ 0.0002 mg/kg for S-1563.