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R182281

R182281 - Method GRM005.17A for the Determination of R182281 in OVS Silica Gel Air Sampling Tubes By LC-MS/MS

Analytical Method

VOLUME 1 OF 1 OF STUDY

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical Method GRM005.17A is suitable for the determination of R182281 (Figure 1) also identified as SDS3701 and hydroxy-chlorothalonil (HCTN) in OVS silica gel sorbent (520 mg/260 mg) air sampling tubes (SKC Inc. Cat No. 226-99). The limit of quantitation (LOQ) of the method has been established at 0.01 µg/OVS (520/260) silica gel air sampling tube.

This method satisfies US EPA 850.6100 guidelines.

1.2 Method Summary

OVS (520/260) silica gel air sample tube contents are extracted by sonication with acidified (1% formic acid) acetone. An aliquot of sample is evaporated to near dryness then reconstituted to final volume with ultra-pure water:acetonitrile (50:50 v/v). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

The limit of quantitation of the method is 0.01 µg/air sample type.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear appropriate PPE which includes chemical resistant gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare a 100 µg/mL stock solution for R182281 by one of the following methods:

Weigh out accurately, using a five figure balance, sufficient R182281 analytical standard into an amber "Class A" volumetric flask (100-mL). Dilute to the mark with methanol and mix well to give a 100 µg/mL stock solution of R182281. Standards should be prepared in amber bottles and stored under refrigeration.

Alternatively, the appropriate volume of solvent added to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P	=	Standard purity in decimal form (P(%)/100)
V	=	Volume of solvent required
W	=	Weight, in mg, of the solid analytical standard
C	=	Desired concentration of the final solution, (µg/mL)
1000	=	Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solutions should be prepared by serial dilution with methanol. It is recommended that the following solutions are prepared: 10 µg/mL, 2.0 µg/mL and 0.20 µg/mL for fortification purposes.

2.3.3 Preparation of Calibration Standards

No significant matrix effects, suppression or enhancement of the instrument response has been observed in the filter types tested using the procedures described in Section 3 during method development and non-matrix matched calibration standards should normally be used for quantitation using LC-MS/MS.

A calibration curve should be generated to quantify R182281. Standards over an appropriate concentration range should be prepared with a minimum of five levels using the

recommended standard range 0.20 µg/L – 10 µg/L (2 pg to 100 pg on column using a 10µL injection) in ultra-pure water:acetonitrile (50:50 v/v).

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2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in amber bottles and refrigerated when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of six months for R182281 is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S. G. Luxon, The Chemical Society, London (Reference 1).

Solvent and Reagent hazards

	Acetonitrile	Methanol	Formic Acid
Harmful Vapor	✓	✓	✓
Highly Flammable	✓	✓	✖
Harmful by Skin Absorption	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓
Causes severe burns	✖	✖	✓
OES Short Term (mg/m^3)	105	310	N./A
OES Long Term (mg/m^3)	70	260	9

N/A not known

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

A summary of the method is included in flow-chart form as shown in Appendix 4. In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included in each sample set. At least one untreated control and two control samples fortified with known amounts of R182281 should be analyzed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

3.1 Sample Preparation

All samples should be prepared/collected using an approved method of preparation to minimize any possible cross-contamination from sample to sample. Evaluation of potential compound break-through should be determined using the specific air sample type identified in the study. Tandem configuration may be required depending on the expected residue level. In preliminary experiments, no break-through was observed using OVS silica gel (520/260) air sampling tubes (SKC Inc. Cat. No. 226-99) for a period of 24 hours under laboratory hood conditions at an in-flow rate of 1Liter/minute.

3.2 Sample Fortification

In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included with each sample set. To each air sample, add the appropriate amount of standard solution (10-50µL recommended) containing R182281 in methanol. Let each sample stand for at least five minutes after fortification to allow the spiking solvent to penetrate/evaporate before proceeding with the extraction procedure. At least one untreated control and two fortified control samples should be analyzed with each sample set.

3.3 Extraction

1. Remove all contents (media, filter, foam plug, plastic holder…etc.) of air sample by pushing forward from small opening to large opening with a glass rod or suitable implement directly into appropriately size vessel (50 mL polypropylene tube). To avoid contamination from the exterior surface of the air sampler, the glass OVS tube should not be included in the extraction.
2. Extract R182281 residue from the sample contents using 10 mL of acidified acetone (1% formic acid).
3. Sonicate sample for 10 minutes.
4. Transfer 0.5 mL of extract to LC autosampler vial or other vessel along with 0.1 mL ultra-pure water to act as a keeper, evaporate to near dryness. (Note: evaporation to complete dryness may result in lower recoveries due to sample loss)
5. Reconstitute sample to 1 mL final volume using ultra-pure water:acetonitrile (50:50 v/v), vortex well.
6. Analyze by LC-MS/MS (ESI Negative Mode).

3.4 Time Required for Analysis

The methodology is normally performed with a batch of 25 samples per set. One skilled analyst can complete the analysis of 1 to 2 sets in 1 day (8 hour working period).

3.5 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekends unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate

any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

3.4 Problems and Modifications

If matrix effect is present, the use of internal standard is recommended.

Proper lab techniques should be implemented to prevent possible contamination of samples, labware and instrumentation.

4.0 FINAL DETERMINATION

4.1 Instrument Description (LC-MS/MS)

UPLC System	:	Acquity UPLC
Detector	:	Sciex API 4000

4.2 Chromatography Conditions

Column	:	ACE 3 C18 3 x 50mm, 3µ
Column Oven Temperature	:	30°C
Injection volume	:	10 µL
Stop Time	:	5.0 minutes
Injection protocol	:	Analyze calibration standard after 5 sample injections
Mobile phase	:	Solvent 1 = 0.1% Formic Acid in Optima Water Solvent 2 = 0.1% Formic Acid in Optima Acetonitrile

Mobile Phase Composition

Time (min)	0.01% Formic Acid in Water	0.01% Formic Acid in ACN	Flow rate, mL/min
0.0	70	30	1.0
0.5	70	30	1.0
2.5	10	90	1.0
3.5	10	90	1.0
4.0	70	30	1.0
5.0	70	30	1.0

Under these conditions the retention time of R182281 is 1.3 minutes.

4.3 Mass Spectrometer Conditions (LC-MS/MS)

Interface	:	ESI
Ionization mode	:	Negative
Curtain gas (CUR)	:	Nitrogen set at 20 (arbitrary units)
Source temperature (TEM)	:	650 °C
Ionspray Voltage (IS)	:	-4200
Collision gas setting (CAD)	:	Nitrogen set at Medium (arbitrary units)
Gas 1 (GS1)	:	Air set at 50 (arbitrary units)
Gas 2 (GS2)	:	Air set at 50 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

MRM Conditions		**R182281 Primary**	**R182281 Confirmatory**
Q1 *m/z*	:	244.8	244.8
Q3 *m/z*	:	181.8	174.8
Dwell time	:	50 ms	50 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	-60 V	-60 V
Entrance potential (EP)	:	-10 V	-10 V
Collision energy (CE)	:	-40 V	-40 V
Collision cell exit potential (CXP)	:	-10 V	-10 V

4.4 Confirmatory Procedures for R182281

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi Point Calibration Procedure

R182281 residues may be calculated in µg/sample for each sample as follows:

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 30% LOQ to 20 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least five levels). And combined with an internal standard to normalize data.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to respective target ions. Quality Control standard solutions should be interspersed throughout the analysis to monitor any matrix effects.

c) Generate calibration curve parameters using an appropriate linear regression package.

d) The linear regression equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient (slope) of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the coefficient of determination, $\mathbf{r^2}$ and correlation coefficient, **r**, for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Calculate residues of interest in a sample, expressed as µg/sample, as follows:

$$\text{Residue (ug/sample)} = \frac{\text{Analyte found } (\mu g/mL)}{\text{Sample Vol. (mL)}}$$

Where analyte found (µg/mL) is calculated from the standard calibration curve and sample vol. is the final sample dilution in mL.

f) Determine the recovery by first subtracting the residue found in the control sample, if any, from the residue found in the recovery sample. Calculate the recovery as a percentage (%) by the equation:

$$Recovery\,(\%) = \frac{(Residue\ in\ Recovery\ Sample) - (Residue\ in\ Control)}{Amount\ Fortified} \times 100\%$$

g) If residues need to be corrected for average percentage recovery, *e.g.* for storage stability studies, then the equation below should be used.

$$Corrected\ Residue = \frac{Residue\ x\ 100}{Average\ percentage\ Recovery}(mg/kg)$$

5.2 Single Point Calibration Procedure

R182281 residues may be calculated in µg/sample for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing R182281 at an appropriate concentration operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for R182281.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to R182281.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the R182281 residues in the sample, expressed as µg/sample using a mean standard response from each of the injections bracketing the sample as follows:

$$\text{Residue (ug/sample)} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Vol.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (µg/mL)
Sample Conc. = Sample volume (mL)

e) If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$Corrected\ Residue = \frac{Residue\ x\ 100}{Average\ percentage\ Recovery}(mg/kg)$$

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analyzed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analyzed with each batch of samples. Control samples from the same matrix are recommended to monitor any instrumental matrix effects present.

At least two recovery samples (control samples accurately fortified with known amounts of R182281), including one at the method LOQ and one at the expected residue level, should also be analyzed alongside each set of samples. The fortification levels should be appropriate to the residue levels expected in the sample.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 120% and with a relative standard deviation of $\leq$ 20%.

When the method is used for monitoring purposes, control and recovery samples are not required where suitable control samples are not available.

7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if potential laboratory contamination is a concern or suspected.

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Interferences arising from the matrices tested have not been observed.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware Interference

This method uses mainly disposable labware. All reusable glassware should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

8.0 METHOD EVALUATION

8.1 Recovery Data and Repeatability

Method validation and associated ILV have been carried out on the procedures described in Sections 3 and 4. The method validation data are summarized in Tables 1 and 2, Independent laboratory validation data summarized in tables 3 and 4.

8.2 Limit of Quantitation (LOQ)

The limit of quantitation of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-120% with a relative standard deviation of $\leq$ 20% has been obtained. Generally, for accurate quantitation, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantitation of the method has been established at 0.01 µg/air sample tube.

8.3 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise. Note that the LOD may vary between runs and from instrument to instrument. The LOD was determined to be 0.20 pg/µL, equivalent to 2 pg on column when using a 10 µL injection volume by LC.

8.4 Matrix Effects

No significant matrix effects were observed in the air sample type tested. Non Matrix-matched standards should generally be used for quantitation. A summary of the matrix effects are included in Table 5.

8.5 Detector Linearity

For accurate quantitation of residue concentrations, analyses should be carried out within the linear range of the detector. For multi-point calibration, detector range and linearity will be demonstrated within each sample set.

The linearity of the LC-MS/MS detector response for R182281 was tested in the range from 2.0 pg to 100 pg injected on column (equivalent to 0.20 pg/µL to 10 pg/µL standards when using a 10 µL injection volume) and was found to be linear. This is equivalent to 20% LOQ - 200% 10XLOQ.

If a residue beyond the tested concentration range is expected, dilute the sample appropriately to bring it within the tested linear range prior to quantitation.

Detector linearity graphs are presented in the Figures Section.

8.6 Final Extract Stability

Final extracts in ultra-pure water:acetonitrile (50:50 v/v) retained in vials and stored at a temperature of approximately 4°C were suitable for R182281 residue analysis, for storage periods of up to 7 days. A summary of the stability data obtained during method validation is presented in Table 6.

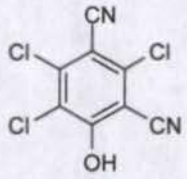
9.0 LIMITATIONS

The method has been tested on representative OVS silica gel (520/260) air sampling tubes (SKC Inc. Cat. No. 226-99). For other samples types the method would require modification and validation using weathered samples and manual fortifications before proceeding with sample analysis.

CHEMICAL STRUCTURES

FIGURE 1 R182281

Compound Code Number	:	R182281
Alternative compound code number	:	SDS3701
CAS Number	:	28343-61-5
IUPAC Name	:	2,4,5-Trichloro-6-hydroxy-isophthalonitrile
Molecular Formula	:	$C_8 H Cl_3 N_2 O$
Molecular Weight	:	247.46



APPENDIX 1 Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General lab glassware	General lab glassware	www.thermoscientific.com
General lab plastic-ware	General lab plastic-ware	www.thermoscientific.com
OVS Silica Gel Tube	SKC Cat No. 226-99	www.skcinc.com
LC Column	ACE 3 C18 3 x50 mm 3 µm	www.ace.com

APPENDIX 2 Reagents

Recommended Suppliers

Reagent	Description	Supplier
Solvents	HPLC grade	www.thermoscientific.com
R182281 analytical standards	GLP certified	Syngenta Crop Protection, LLC

Preparation of Reagents

a) Acidified Acetone (1% Formic Acid): 10 mL Reagent Grade Formic Acid to 990 mL ACS Grade acetone.

b) Ultra-pure water: acetonitrile (50:50 v/v): 500 mL of ultra-pure water added to 500 mL ACS Grade Acetonitrile.

APPENDIX 3 LC-MS/MS Tuning Procedure

Calibration of Instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning Instrument for R182281

Infuse standard solutions of R182281 (0.1 to 1.0 µg/mL) in mobile phase (see section 4) directly into the mass spectrometer interface at a rate at of approximately 10-20 µL/min. Roughly adjust interface parameters (sprayer position, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at *m/z* 244 for R182281 in negative ionization mode.

Using the Analyst software quantitative optimisation routine, tune the instrument for R182281, ensuring that the correct ion is selected. If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of a R182281 standard using mobile phase at the flow rate to be used. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.

For R182281, in negative ionization mode, the deprotonated molecular ion generated in the ion source (*m/z* 244) is selected and subjected to further fragmentation by collisional activation. The two most sensitive daughter ions (*m/z* 181 and *m/z* 174) are then selected and used for quantitative analysis.

APPENDIX 4 Method Flow Chart

Transfer contents of OVS tube into a 50 mL poly tube

↓

Add 10 mL acidified acetone (1% formic acid)

↓

Sonicate sample for 10 minutes

↓

Evaporate 0.5 mL with keeper to near dryness

↓

Reconstitute to1 mL using ultra-pure water:acetonitrile (50:50 v/v) and vortex

↓

Analyze by LC-MS/MS