

SUMMARY

An analytical method, employing gas chromatography with electron capture detection (GC/ECD), for the determination and confirmation of OK-5203 in drinking water and surface water was validated at Covance Laboratories Limited.

Characteristics of the test systems are shown below.

Water type	Source	pH	DOC (mg/L)	Total hardness (mg/L as CaCO ₃)	Conductivity (µS/cm)	Silt as suspended solids (mg/L)	Nitrate as N (mg/L)	Chloride (mg/L)
drinking (tap)	Test Facility AH 7.4	7.48	-	70.4	203	-	<1.15	18.8
surface (lake)	Fountains Abbey, North Yorkshire, UK	7.3	18.16	110	362	0.3	-	-

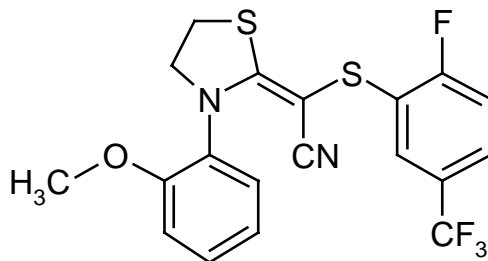
DOC = dissolved organic carbon

The method involved extraction from drinking water or surface water with toluene prior to determination of OK-5203 by GC/ECD.

Control samples of drinking water and surface water were fortified with OK-5203 at 0.1 and 1.0 µg/L, in quintuplicate and analysed. Recovery of OK-5203 was determined and the validity of the analytical procedure was assessed.

INTRODUCTION

OK-5203 is a development fungicide and has the following chemical structure:



Chemical Name (IUPAC): (Z)-2-[3-(2-methoxyphenyl)-2-thiazolidinylidene]-2-(α, α, α , 6-tetrafluoro-*m*-tolylthio)acetonitrile

CAS Registry Number: [304900-25-2]

Chemical Formula: $C_{19}H_{14}F_4N_2OS_2$

Molecular Weight: 426.45

The study was undertaken to comply with the known data requirements and study guidelines stated on the front cover of this report. These requirements will satisfy EU post registration enforcement method validation. An analytical method validated to these guidelines is considered suitable for regulatory submission in areas outside of the EU, e.g. USA.

OBJECTIVES

The objective of this study was to develop and validate an analytical method, using gas chromatograph determination with either electron capture (ECD) or mass selective (MSD) determination, for measuring residues of OK-5203 in drinking water and surface water.

PROCEDURES

Protocol Adherence

The study was performed in accordance with the protocol, with no deviations.

Analytical Standards

Analytical grade OK-5203 was supplied by the Sponsor. A Certificate of Analysis including purity, expiry date and batch number was supplied.

All precautions required in the handling, storage and disposal of the test substance were outlined by the supplier.

The date of receipt and expiry date, plus batch number and purity details are recorded in the table below along with the allocated Covance identification and lot number. The analytical standard was stored under refrigeration (1 to 10°C, nominally 4°C). The Certificate of Analysis is presented in Appendix 1.

Analytical standard	Covance CHD number, (lot number)	Date received	Supplier Batch no.	Chemical purity	Expiry date
OK-5203	350/05-2554 (3)	11 May 2006	04AF1-1	99.13%	28 June 2007

Receipt and Storage of Study Samples

Control samples of drinking water (Test Facility tap water) and surface water (lake water) were sourced locally by Covance.

The samples were given unique Covance residue numbers on receipt (06/2554/306 for drinking water and 06/0000/277 for surface water) in accordance with departmental Standard Operating Procedures (SOPs), and were stored under refrigeration (1 to 10°C, nominally 4°C). The surface water system was also assigned the unique code CS40/06 for the characterisation tests.

Water Characterisation

Water characteristics are presented in Appendix 2. A summary of these data is shown below.

Water type	Source	pH	DOC (mg/L)	Total hardness (mg/L as CaCO ₃)	Conductivity (µS/cm)	Silt as suspended solids (mg/L)	Nitrate as N (mg/L)	Chloride (mg/L)
Drinking (tap)	Test Facility AH 7.4	7.48	-	70.4	203	-	<1.15	18.8
Surface (lake)	Fountains Abbey, North Yorkshire, UK	7.3	18.16	110	362	0.3	-	-

DOC = dissolved organic carbon

Preparation of Study Samples

Control samples of drinking water and surface water did not require any preparation prior to analysis.

Analytical Procedures

Analytical procedure CLE 2554/020-01V was developed by Covance. The analytical method employed gas chromatography with electron capture determination (GC/ECD). GC/ECD was selected rather than GC/MSD since it provided increased sensitivity of data.

Summary of the Analytical Method for Drinking Water and Surface Water. Method CLE 2554/020-01V

The method involved extraction by direct partition into organic solvent (toluene) from the water matrices. Determination of OK-5203 was by GC/ECD.

A copy of the analytical procedure is presented in Appendix 3.

Validation Procedure

The analytical method was validated by fortifying aliquots of untreated control drinking water and surface water with known amounts of OK-5203. The fortified samples were then analysed using the defined method and recovery of OK-5203 from each sample was determined.

Validation included consideration of the criteria in the following sections.

Linearity

In order to establish linearity of response of the analytical chromatographic system to OK-5203, at least six standard solutions of increasing concentration were prepared over the range 0.001 to 0.1 µg/mL for validation of drinking water and surface water. The lowest concentration was equivalent to less than 50% of a sample at the limit of quantification (LOQ) and the highest concentration was equivalent to greater than 120% of the highest level to be analysed.

Solutions were injected into the chromatograph in random order, and concentration/response curves were prepared.

Specificity

The ability of the method to distinguish between OK-5203 and other substances present in the control samples was investigated. Components present in a control sample that interfered with the analysis should not have been present at levels greater than 30% of the limit of quantification.

Precision

Repeatability of the method was demonstrated by analysing each validation level in quintuplicate. Relative standard deviation (RSD) was determined at each validation level and was considered acceptable if $\leq 20\%$.

Recovery

Recovery from control drinking water and surface water fortified at 0.1 (LOQ) and 1.0 µg/L (10 x LOQ) of OK-5203 was determined in quintuplicate.

In addition, control samples of drinking water and surface water were extracted and analysed in duplicate.

Where there was an apparent response to OK-5203 from a control matrix, the equivalent concentration was subtracted from each of the relevant matrix validation levels before calculation of the recovery values.

Mean recoveries of OK-5203 at each level and overall, were considered acceptable if they fell within the range 70 to 110%.

Limit of Quantification (LOQ)

The limit of quantification was defined as the lowest fortification level where an acceptable mean recovery for OK-5203 was obtained (70 to 110%), and a relative standard deviation of $\leq 20\%$ was achieved. For this analytical method, the limit of quantification for OK-5203 was proposed as 0.1 $\mu\text{g/L}$ in both drinking water and surface water.

Limit of Detection (LOD)

Although not specifically required according to the EU guidance documents stated on the front cover of this report, an analytical LOD was estimated for each matrix. The limit of detection was defined as the lowest concentration of the test compound measurable in each water matrix extract, estimated from the lowest concentration of the calibration standards. The LOD should be 30% of the LOQ or lower, and its signal to noise ratio (S/N) should be greater than three.

Method Confirmation

Since the selected method did not involve determination using GC/MS (3 ions), re-analysis of final extracts, using a suitably selective and sensitive alternative chromatographic technique, was required. The same criteria for precision and recovery (see above) were used to assess this confirmatory method.

SAFETY PRECAUTIONS

Operators should take the normal precaution of wearing gloves, laboratory coats and safety glasses when handling compound and matrix samples.

Safety assessments (Control of Substances Hazardous to Health, COSHH) have been made of those procedural steps involving preparation of solutions, reagents and analysis of matrix samples. Appropriate safety codes have been included in the text (e.g. Category [1a/b, 4b/4d]) and these are defined in the section titled General Handling Control Categories (on page 35 of this report).

The hazards and risks of the substances hazardous to health used in this method have been considered. Provided the method is accurately followed and the control measures specified in the method are correctly used, there should be no foreseeable hazards to health.

INTRODUCTION

This method describes the procedure for determining residues of OK-5203 in drinking water and surface water. Residues of OK-5203 in drinking water and surface water are extracted by using liquid-liquid partition into toluene. OK-5203 is quantified by GC using ECD detection. The limit of quantification for this procedure is 0.1 µg/L.

Summary of the Procedure

The method of analysis comprises the following stages:

1. Extraction from drinking water and surface water by liquid-liquid partition into toluene.
2. Quantification by GC/ECD detection.

All procedures will be carried out in compliance with departmental SOP.

APPARATUS, MATERIALS, REAGENTS AND SOLUTIONS

Apparatus and Glassware

- GC-ECD system: Agilent 6890 series GC system
- GC Column: HP5, 30m x 0.32 mm i.d, 0.25 µm
- Centrifuge MSE Mistral 3000E
- 50 mL Polypropylene tubes-Costar
- 2 mL GC autosampler vials with snap-on caps
- Various laboratory glassware
- Various pipettes
- Short form pipettes

Equivalent equipment may be used

Materials

- Toluene Rathburn, Glass distilled grade
- Acetone Rathburn, Glass distilled grade

Equivalent or better grade reagents/solvents may be used.

Preparation of Standard Solutions [Category 1a/b, 4b/4d]

Primary stock standard solutions of OK-5203 are prepared in acetone. Diluted fortification solutions are prepared in acetone. Standard solutions must be stored refrigerated in glass when not in use. Solutions should be allowed to warm to room temperature prior to use.

Preparation of Stock Solutions [Category 1a/b, 4d]

In duplicate, accurately weigh *ca.* 10 mg (corrected for purity) of OK-5203 into separate 10 mL volumetric flasks and dilute each to the mark using acetone to give primary stock standards of concentration 1000 µg/mL.

Note - Duplicate solutions of each test substance are prepared to check both the accuracy of weighing and solubility of the test substances. Correlation is achieved by GC/ECD quantification of appropriately diluted solutions. Only one primary stock standard is used for the preparation of both fortification and calibration solutions.

Calibration Standards [Category 1a/b, 4b]

Prepare appropriate calibration standards in toluene (a minimum of six concentrations) to cover the range 0 to 0.1 µg/mL, with a lowest calibration level of 0.001 µg/mL. Calibration standards may be stored refrigerated for up to one month.

Fortification Solutions [Category 1a/b, 4b]

Prepare suitable fortification solutions in acetone by serial dilution from the primary stock solution (1000 µg/mL in acetone). Fortification standards may be stored refrigerated for up to one month.

PROCEDURES

All work should be carried out under the minimum control categories listed under the safety precautions section. Additional controls are listed with the individual steps of the procedure.

Extraction From drinking water and surface water [Category 1a/b, 4a]

1. Place 50 mL of either drinking water or surface water into a 50 mL polypropylene tube. Untreated control samples may be fortified at this point for the determination of procedural recovery (with up to 500 µL of a suitable fortification solution in acetone).
2. Add 2 mL of toluene
3. Secure cap on tube and shake (by hand) vigorously for 1 minute ± 5 seconds.
4. Centrifuge the tubes at 2500rpm (1400 g) for 5 minutes.
5. Remove as much of the toluene layer as possible without disturbing any emulsion interface and transfer into a GC autosampler vial ready for determination by GC/ECD
6. Further dilution with toluene within the calibration range can be made at this point, or subsequently, if required.

All extracts derived during or from this procedure may be stored refrigerated or frozen if storage is required.

Conditions for GC Analysis with ECD Detection

Instrumentation:	Agilent 6890 series GC µECD system and 7683 series autosampler
Column#:	J&W Scientific, HP5, 30 m x 0.32 mm x 0.25 µm film
Injection Volume:	1 µL, split 1:5
Carrier gas:	Helium
Make up gas:	Nitrogen
Head Pressure:	20 psi
Column temperature:	
Injection temperature:	90°C hold for 0.2 minutes, ramp 700°C per minute to 310°C hold 5 minutes, ramp 50°C per minute to 290°C
Detector temperature:	300°C
Retention time:	8.1 minutes

Parameters marked # may not be modified. Minor adjustments to the remaining parameters may be required in order to fully optimise the system.

Confirmation Conditions for GC Analysis with ECD Detection

Instrumentation:	Agilent 6890 series GC μ ECD system and 7683 series autosampler
Column#:	J&W Scientific, DB17, 15 m x 0.25 mm x 0.25 μ m film
Injection Volume:	1 μ L, split 1:5
Carrier gas:	Helium
Make up gas:	Nitrogen
Head Pressure:	20 psi
Column temperature:	
Injection temperature:	90°C hold for 0.2 minutes, ramp 700°C per minute to 310°C hold 5 minutes, ramp 50°C per minute to 290°C
Detector temperature:	300°C
Retention time:	8.1 minutes

Parameters marked # may not be modified. Minor adjustments to the remaining parameters may be required in order to fully optimise the system.

CALCULATION OF RESULTS

The presence of OK-5203 in a sample is confirmed if the resulting peak arising from the test sample has the same chromatographic retention times as the appropriate standard. All peak measurements and calculations are performed using a data system (ATLAS 2000R2).

Residues of OK-5203 are determined by the interpolation of the peak area of OK-5203, from the standard regression equation, as follows:

The calibration line is determined by plotting the responses from the calibration solutions (R) against the amount of test substance injected (A) to generate a straight line graph.

$$R = B_0 + B_1 \times A$$

where B₁ is the gradient and B₀ is the intercept.

Concentrations of test substance (A) in sample extracts are calculated from their response using the equation:

$$\text{Concentration of extract A } (\mu\text{g/mL}) = (\text{peak area} - \text{intercept})/\text{gradient}$$

The use of weighted least squares regression (1/x) for the calculation of intercept and gradient is recommended for calibration lines with a range in excess of 100. Standard linear regression and weighted least squares regression (1/x) calculations are performed using suitable validated software.

The residue of OK-5203 in each test sample is calculated as follows:

$$\text{Residue } (\mu\text{g/L}) = \frac{\text{extract concentration } (\mu\text{g/mL}) \times \text{final volume (mL)}}{\text{sample volume (L)}}$$

Where the final volume includes dilution steps, if applicable.

Procedural recovery data from fortified samples are calculated via the following equation:

$$\text{Recovery } (\%) = \frac{A - C}{S} \times 100$$

Where:-

A = concentration found in fortified sample (μg/L)

C = concentration (or interference) found in control sample (μg/L)

S = concentration added to fortified sample (μg/L)

Linearity of Response

The linearity of response of the GC system should be determined with at least six different concentration standard solutions across the range 0 to 0.1 $\mu\text{g/mL}$, with the lowest calibration level being 0.001 $\mu\text{g/mL}$.

METHOD CRITERIA

Analysis by GC will be considered successful only if the following criteria are met.

- A procedural recovery of 70 to 110% is obtained for each batch of analysis
- Control sample contains a concentration $\leq 30\%$ of the limit of quantification
- At least 6 calibration standards are used in the determination of each calibration line
- Using a 1/x weighing, the coefficient of determination (r^2) for each calibration line is ≥ 0.98
- All test samples are within the appropriate calibration standards range

GENERAL HANDLING CONTROL CATEGORIES

CATEGORY		CONTROL
Main	Division	Name and Specification
1	a b c d	GLOVES Disposable latex Disposable nitrile Rubber gloves Specific type for the job (see assessment giving details)
2	a b c d e	PROTECTIVE CLOTHING Laboratory coat or equivalent Disposable overalls Oversleeves Overshoes Plastic apron
3	a b c	EYE/FACE PROTECTION Safety glasses to BS 2092/2 C or better Face shield to BS 2092/2 C or better Safety goggles to BS 2092/2 C or better
4	a b c d e f g	ENGINEERING CONTROLS Open bench in ventilated area Fume cupboard to BS 7258 Laminar flow cabinet to BS 5295 Class 1 Re-circulating fume chamber Radioisotope lab Biohazard lab Glove box
5	a b c	RESPIRATORY PROTECTIVE EQUIPMENT Disposable filtering facemask (HSE approved), i - organic vapour ii - dust iii - combination organic vapour/dust MUST SPECIFY TYPE b Powered respirators/helmets with safety visor to BS 2092/2 C or better (HSE approved) c Respirator with specified canister (HSE approved)
6		SPECIFIC IMMUNISATION REQUIRED (GIVE DETAILS)
7		ALLERGIC PERSONS PROHIBITED (SPECIFY ALLERGY)
8		REFER TO MATERIAL SAFETY DATA SHEET
9		KNOWN OR SUSPECTED REPRODUCTIVE HAZARD TO EITHER SEX (must specify details)
10		POISON – ensure antidote is available and is within its expiry date (must specify details)