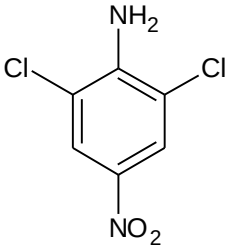
MATERIALS AND METHODS

Protocol Adherence
The study was conducted in accordance with the protocol with no deviations.

Test Substance
The following test substance was used to fortify the samples, as per the analytical method validated by Smithers ERS (Wareham):

Test Substance Name:	**Dicloran Technical**
IUPAC Name:	2,6-Dichloro-4-nitroaniline
CAS Number:	99-30-9
Structure:	
Molecular Formula:	$C_6H_4Cl_2N_2O_2$
Molecular Weight:	207.0142 g/mol
Sponsor Lot Number:	20130605
Purity:	98.9%
Storage Conditions:	Room Temperature (15-25°C)
Recertification Date:	19 April 2021

The Certificate of Analysis for the test substance is presented in Appendix 1.

Test Matrices
Control sandy loam and silt loam soil were sourced by Smithers ERS. The soils used were CS 30/18 (sandy loam) and CS 17/18 (silt loam).

Soil characterisation data are listed in the following table:

Soil Name	Textural class[1]	% Sand, Silt, Clay[2]	CEC (meq/100 g)	% Organic Carbon	pH in H_2O	pH in 0.01M $CaCl_2$
Refe Sol 01-A	sandy loam[3]	74, 20, 6[3]	5.3	0.9[3]	6.4	5.3[3]
Newhaven	silt loam	25, 51, 24	17.4	3.2	6.0	5.4

[1, 2] USDA classification.
[3] Soil characterisation data provided by Fraunhofer.

The certificates of analysis for each soil are presented in Appendix 2.

The moisture contents of the soils were determined to be 2.41% and 27.29% of the dry soil weight for Refe Sol 01-A and Newhaven soil respectively.

Reagents

• Acetonitrile	HPLC grade, Honeywell
• Water	Milli-Q (with LCPAK polisher)
• 0.1% Formic acid in water	LC-MS grade, Honeywell
• 0.1% Formic acid in acetonitrile	LC-MS grade, Honeywell

Equipment

- Shimadzu Nexera series HPLC system with AB Sciex API 5000 MS/MS detector.
- HPLC column: Phenomenex kinetex 2.6 µm phenyl-hexyl 3 × 50 mm
- Analytical balance
- Centrifuge
- Centrifuge tubes
- Glass jars
- Orbital shaker: Edmund Buhler SM 30 A
- Positive displacement pipettes
- Volumetric flasks
- Amber glass vials
- Disposable glass vials
- HPLC vials

Analytical Method

Analytical method 12791.6320 was supplied by Smithers ERS, Wareham on behalf of the sponsor. The method was re-written in Smithers ERS, Harrogate format as draft method SMI 3202456-01D, including the instrumentation available at Smithers ERS, Harrogate. This was used for method validation, and re-issued as SMI 3202456-01V when validation was complete. The complete analytical procedure is presented in Appendix 3.

Preparation of Reagents

Acetonitrile: water (20:80 v/v)

Acetonitrile: water (20:80 v/v) was prepared by mixing 100 mL HPLC grade acetonitrile with 400 mL Milli-Q water.

Reagents were stored at room temperature and given a nominal expiry date of one month.

Preparation of Stock Solutions

Primary Stock Solutions

Primary stock solutions of Dicloran were prepared at 1000 µg/mL in acetonitrile under Smithers ERS GLP Study No. 3202455 (Independent Laboratory Validation of Analytical Method 12791.6319 for the determination of Dicloran in Ground Water and Surface Water by LC-MS/MS). Primary stock solutions were stored refrigerated for up to 3 months.

Secondary stock solutions

Secondary stock solutions of Dicloran were prepared at 10 µg/mL in acetonitrile under Smithers ERS GLP Study No. 3202455 (Independent Laboratory Validation of Analytical Method 12791.6319 for the determination of Dicloran in Ground Water

and Surface Water by LC-MS/MS). Secondary stock solutions were stored refrigerated for up to 1 month.

Sub-Stocks
Sub-stock solutions of Dicloran in acetonitrile were prepared as described in the following table.

Secondary Stock Concentration (µg/mL)	Volume Taken (mL)	Solvent	Final Volume (mL)	Sub-Stock Concentration (µg/mL)
10	1	Acetonitrile	10	1
10	0.1		10	0.1[1]

[1] Equivalent to 100 µg/L.

Sub-stock solutions were prepared on the day of the use and stored refrigerated until the corresponding analysis was complete.

Preparation of Matrix Matched Standards for Matrix Assessment
Matrix matched standards of Dicloran were prepared in control soil final extract as shown in the table below.

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.1	Sandy loam soil final extract	10	1
100	0.1		10	1
100	0.1		10	1
100	0.1	Silt loam soil final extract	10	1
100	0.1		10	1
100	0.1		10	1

Preparation of Non-Matrix Matched Standards for Matrix Assessment
Non-matrix matched standards of Dicloran were prepared in acetonitrile: water (20:80 v/v) for comparison with matrix matched standards as described in the following table.

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.1	Acetonitrile: water (20:80 v/v)	10	1
100	0.1		10	1
100	0.1		10	1

The three matrix matched standards for each soil were analysed with the three non-matrix matched standards and their peak areas compared.

Preparation of Calibration Standards

Non-matrix matched calibration standards of Dicloran were prepared for the validation of sandy loam soil as described in the following table:

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.5	Acetonitrile: water (20:80 v/v)	10	5
5	0.8		1	4
5	0.6		1	3
5	0.4		1	2
5	0.2		1	1
5	0.15		1	0.75
5	0.1		1	0.5

Matrix-matched calibration standards of Dicloran were prepared for the validation of silt loam soil as described in the following table:

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.5	Control silt loam final extract	10	5
5	0.8		1	4
5	0.6		1	3
5	0.4		1	2
5	0.2		1	1
5	0.15		1	0.75
5	0.1		1	0.5

A single set of calibration standards was prepared for each validation batch, which was analysed twice during the batch, interspersed with the samples.

Sample Preparation and Fortification

5±0.05 g dry weight equivalent of soil was weighed into a Polypropylene centrifuge tube. Quintuplicate soil samples were fortified at the LOQ (50 µg/kg) and at 10 × LOQ (500 µg/kg) with a stock solution of Dicloran. Duplicate control soil samples and a reagent blank (acetonitrile) were also prepared, as described in the following tables:

Sandy loam soil

Sample ID	Sample Weight (g)	Stock Concentration (µg/mL)	Volume Added (mL)	Fortified Concentration (µg/kg)
Reagent Blank A[1]	N/A	N/A	N/A	N/A
Control A[2]	5	N/A	N/A	N/A
Control C-D	5	N/A	N/A	N/A
F0.05 A-E	5	1	0.25	50
F0.5 A-E	5	10	0.25	500

N/A = Not applicable.
[1] No soil was used for the reagent blank.
[2] Control A was used for matrix assessment.

Silt loam soil

Sample ID	Sample Weight (g)	Stock Concentration (µg/mL)	Volume Added (mL)	Fortified Concentration (µg/kg)
Reagent Blank B[1]	N/A	N/A	N/A	N/A
Control B[2]	5	N/A	N/A	N/A
Control E-F[3]	5	N/A	N/A	N/A
F0.05 F-J	5	1	0.25	50
F0.5 F-J	5	10	0.25	500

N/A = Not applicable.
[1] No soil was used for the reagent blank.
[2] Control B was used for matrix assessment.
[3] Control sample was used to dilute samples and prepare matrix matched calibration standards.

Sample Extraction

The samples were extracted twice with 20 mL acetonitrile by sonicating for 10 minutes and shaking at 250 rpm for 30 minutes and centrifuging at 3000 rpm for 10 minutes. The supernatant was transferred into a glass jar and made to 50 mL with acetonitrile in a volumetric flask. The sample extract was diluted into calibration range with acetonitrile: water (20:80 v/v) or control soil final extract for matrix matching. This was centrifuged at 13000 rpm for 5 minutes and transferred into an HPLC vial for analysis. The extraction and dilution procedure is summarised in the following tables:

Sandy loam soil

Sample ID	Fortified Concentration (µg/kg)	Sample Weight (g)	Extract Volume (mL)	Dilution (mL-mL)	Dilution Factor
Reagent blank A	N/A	N/A	50	0.2-1	50
Control A	N/A	5	50	10-50	50
Control C-D	N/A	5	50	0.2-1	50
F0.05 A-E	50	5	50	0.2-1	50
F0.5 A-E	500	5	50	0.04-1	250

N/A = Not applicable.

Silt loam soil

Sample ID	Fortified Concentration (µg/kg)	Sample Weight (g)	Extract Volume (mL)	Dilution (mL-mL)	Dilution Factor
Reagent blank B	N/A	N/A	50	0.2-1	50
Control B	N/A	5	50	10-50	50
Control E-F	N/A	5	50	0.2-1[1]	50
F0.05 F-J	50	5	50	0.2-1	50
F0.5 F-J	500	5	50	0.04-1	250

[1] A 10 mL aliquot of the control sample was diluted 5-fold with acetonitrile: water (20:80 v/v) to prepare matrix-matched calibration standards and for dilution of samples.

Instrument Conditions

LC-MS/MS analysis was performed using the following instrument conditions:

LC Parameters:

Instrument:	Shimadzu Nexera series HPLC system		
Column#:	Phenomenex Kinetex 2.6 µm phenyl-hexyl 3 × 50 mm		
Mobile Phase A#:	0.1% Formic acid in water		
Mobile Phase B#:	0.1% Formic acid in acetonitrile		
Flow Rate:	0.5 mL/min		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	80	20
	0.5	80	20
	3.0	0	100
	4.0	0	100
	4.1	80	20
	5.5	80	20
Run Time:	5.5 minutes		
Column Temperature:	40°C		
Autosampler Temperature:	4°C		
Injection Volume:	50 µL[1]		
Retention Time:	Approx. 2.5 minutes		
Valco Valve Diverter:	Time (min)	Position	
	0	A (to waste)	
	0.5	B (to MS)	
	5.5	A (to waste)	

MS/MS Parameters:

Instrument:	AB Sciex API 5000 Triple Quadrupole Mass Spectrometer		
Ionisation Type#:	Electrospray (ESI)		
Polarity#:	Positive		
Scan Type#:	Multiple reaction monitoring (MRM)		
Ion Spray Voltage:	4500 V		
Collision Gas (CAD):	8		
Curtain Gas (CUR):	20		
Gas Flow 1 (GS1):	40		
Gas Flow 2 (GS2):	40		
Vaporiser Temperature (TEM):	550°C		
Interface Heater (ihe):	On		
Entrance Potential (EP):	10		
Declustering Potential (DP):	50		
Collision Exit Potential (CXP)	25		
Resolution Q1/Q3:	Unit/Unit		
Transition Name:	MRM Transition Ions Monitored	Collision Energy (CE)	Dwell Time (ms)
Dicloran (Primary):	207/190	23	200
Dicloran (Confirmatory):	207/160	35	200

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

[1]10 µL was used as the injection volume for the repeat matrix assessment.

Calculation of Results
When the calibration fit is linear as in this study, Analyst uses the following formula to calculate the concentration of test substance present in the sample:

$$x = \frac{(y - c)}{m} \times DF$$

Where:

x = concentration of test substance in sample extract (µg/L)
y = peak area due to test substance
c = *y* intercept on calibration graph
m = gradient of the calibration graph
DF = sample dilution factor

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

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

Where:-

A = concentration found in fortified sample (µg/kg)
S = concentration added to fortified sample (µg/kg)

The Limit of Detection (LOD) based upon the sample concentration equivalent to three times the baseline noise of a control sample was calculated as follows:

LOD (µg/kg) = 3 × height of control baseline noise × control dilution factor × calibration standard concentration (µg/L) / height of calibration standard peak

The Method Detection Limit (MDL) based upon the sample concentration equivalent to the lowest calibration standard was calculated as follows:

MDL (µg/kg) = lowest calibration standard (µg/L) × control sample dilution factor

Validation Pass Criteria
The validation was deemed acceptable if the following criteria were met for the primary and confirmatory transitions monitored for each compound:

Mean Recovery and Precision
Recovery and precision were acceptable if each fortification level had a mean recovery between 70 and 110% and a % RSD (relative standard deviation) ≤ 20%.

Specificity/Selectivity
Specificity was acceptable if no significant interferences at the retention time of Dicloran were found in the control samples at > 30% of the LOQ peak area response.

Linearity
The linear range was acceptable if the lowest calibration standard concentration was ≤ 80% of the equivalent LOQ final extract concentration. The highest calibration standard concentration was ≥ 120% of the 10 × LOQ extract concentration (after dilution). The correlation coefficient (r) was acceptable if it was ≥ 0.995.

LOD (Limit of Detection) Assessment
An estimate of the LOD was made at 3 × baseline noise for primary and confirmatory transitions for both Dicloran.

MDL (Method Detection Limit)
The MDL was calculated as the initial sample concentration equivalent to the lowest calibration standard (based upon a lowest standard concentration of 0.5 µg/L and a dilution factor of 50).

Matrix Assessment
An assessment of matrix effects was made by comparison of peak areas for triplicate standards prepared in acetonitrile: water (20:80 v/v) and in each control soil final extract. This was assessed for the primary and confirmatory transitions of Dicloran.

Results were presented as a % difference from the mean non-matrix standard value.

A difference of ≥ 20% was considered significant.

If matrix effects were determined to be significant, matrix matched calibration standards would be used for method validation.

REVISION HISTORY

SMI 3202456-01D	New method for independent laboratory validation based upon Smithers ERS, Wareham study 12791.6320.
SMI 3202456-01 V	Re-issued following method validation.

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 and are defined in the section titled General Handling Control Categories.

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 concentrations of Dicloran in silt loam and sandy loam soil by LC-MS/MS. Samples are extracted twice with acetonitrile. An aliquot is diluted into calibration range with acetonitrile:water (20:80 v/v). The extracts are quantified by LC-MS/MS.

Matrix effects for Dicloran in silt loam and sandy loam soil will be determined by comparing peak areas of calibration standards prepared in control soil final extract and in acetonitrile:water (20:80 v/v). Matrix effects are considered significant if the matrix matched standard area is ≥ 20% different from the non-matrix standard area. If matrix effects are significant then matrix matched calibration standards should be used.

APPARATUS, MATERIALS, REAGENTS AND SOLUTIONS

Apparatus and Glassware

- Shimadzu Nexera series HPLC system with AB Sciex API 5000 MS/MS detector.
- HPLC column: Phenomenex kinetex 2.6 µm phenyl-hexyl 3 × 50 µm
- Analytical balance
- Centrifuge
- Centrifuge tubes
- Glass jars
- Orbital shaker: Edmund Buhler SM 30 A
- Positive displacement pipettes
- Volumetric flasks
- Amber glass vials
- Disposable glass vials
- HPLC vials

Equivalent equipment may be used if required

Materials

• Acetonitrile	HPLC grade, VWR
• Water	Milli-Q (with LCPAK polisher)
• 0.1% Formic acid in water	LC-MS grade, Honeywell
• 0.1% Formic acid in acetonitrile	LC-MS grade, Honeywell

Equivalent materials may be used if required

Reagents

Acetonitrile: water (20:80 v/v)

Acetonitrile: water (20:80 v/v) is prepared by mixing 100 mL HPLC grade acetonitrile with 400 mL Milli-Q water.

Reagent volumes may be scaled as appropriate.

Standard Solution Preparation [1b, 4a]

Primary Standard Stock

Prepare duplicate stock solutions of Dicloran at 1000 µg/mL in acetonitrile. Accurately weigh ≥ 10 mg test substance, corrected for purity and transfer into a 10 mL volumetric flask. Adjust the volume to give exactly 1000 µg/mL. Transfer into amber glass bottles. The primary stocks should be stored refrigerated and given a nominal expiry date of 3 months.

Standard Correlation

Dilute the duplicate primary stocks to the mid-point of the calibration line. Correlate the standard solutions by injecting each of the two calibration standards 5 times into the LC-MS/MS. Ensure that the two solutions are injected alternately in the run sequence. The results for the correlation should be ± 5% of the overall mean calculated by peak areas.

Review of Results

Review the data and document the correlation calculations. If the correlation is out of specification, either repeat the injections, re-dilute, or prepare two new stock standards and repeat the procedures in sections *<<Primary Standard Stock>>* to *<<Review of Results>>*.

If the acceptance criteria from section *<<Standard Correlation>>* have been met, then the calibration solutions are acceptable for use. If required, fortification solutions for method validation will be made from the same stock standard, or its dilutions, from which the calibration line has been prepared.

Secondary Standard Stocks

Prepare secondary stock solutions of Dicloran in acetonitrile. The following dilution scheme is suggested:

Primary Stock Concentration (µg/mL)	Volume Taken (mL)	Solvent	Final Volume (mL)	Secondary Stock Concentration (µg/mL)
1000	0.1	Acetonitrile	10	10

Transfer into amber glass bottles. The secondary stocks should be stored refrigerated and given a nominal expiry date of 1 month.

Sub-Stocks

Prepare sub-stock solutions of Dicloran in acetonitrile. The following dilution scheme is suggested:

Secondary Stock Concentration (µg/mL)	Volume Taken (mL)	Solvent	Final Volume (mL)	Sub-Stock Concentration (µg/mL)
10	1	Acetonitrile	10	1
1	1		10	0.1[1]

[1] Equivalent to 100 µg/L.

Transfer into disposable glass vials. The sub-stock solutions should be prepared on the day of use.

Matrix Matched Standards for Matrix Assessment

Prepare silt loam and sandy loam matrix matched standards of Dicloran in disposable glass vials as described in the following table.

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.1	Control soil final extract	10	1
100	0.1		10	1
100	0.1		10	1

Non-Matrix Matched Standards for Matrix Assessment
Prepare non-matrix matched standards of Dicloran in acetonitrile: water (20:80 v/v). The following dilution scheme is suggested:

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.1	Acetonitrile: water (20:80 v/v)	10	1
100	0.1		10	1
100	0.1		10	1

Calibration Standards
Prepare calibration standards of Dicloran in acetonitrile: water (20:80 v/v). The following dilution scheme is suggested:

Stock Concentration (µg/L)	Volume Taken (mL)	Solvent	Final Volume (mL)	Concentration (µg/L)
100	0.5	Acetonitrile: water (20:80 v/v)[1]	10	5
5	0.8		1	4
5	0.6		1	3
5	0.4		1	2
5	0.2		1	1
5	0.15		1	0.75
5	0.1		1	0.5

[1] If matrix matched calibration standards are required, use control soil final extract as the solvent.

A single set of calibration standards should be prepared for each validation batch and injected twice, interspersed with and bracketing the samples.

PROCEDURES

All procedures will be carried out in compliance with departmental SOPs, following departmental safety procedures in conjunction with COSHH assessments.

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.

Fortification of Control Samples for Method Validation [1b, 4a]
Weigh 5±0.05 g dry weight of either silt loam or sandy loam into a polypropylene centrifuge tube. Fortify samples with Dicloran standard in acetonitrile. The following fortification scheme is suggested:

Number of Replicates	Sample Type	Stock Concentration (mg/L)	Volume Added (mL)	Sample Weight (g)	Fortified Concentration (mg/kg)
1	Reagent blank	N/A	N/A	N/A	N/A
2	Control	N/A	N/A	5	N/A
5	LOQ	1	0.25	5	0.05
5	10 × LOQ	10	0.25	5	0.5

N/A = Not Applicable.

Sample Extraction [1b, 4a]

1. Measure 5±0.05 g of soil into a polypropylene centrifuge tube.
2. Add 20 mL acetonitrile to the soil and sonicate for 10 minutes.
3. Shake at 250 rpm for 30 minutes.
4. Centrifuge at 3000 rpm for 10 minutes.
5. Transfer the extracts into a glass jar.
6. Repeat steps 2 to 5, combining the two extracts
7. Make to 50 mL with acetonitrile using a volumetric flask
8. Dilute into the calibration range using acetonitrile:water (20:80 v/v) or control soil final extract if matrix matching.
9. Centrifuge samples at 13,000 rpm for 5 minutes.
10. Transfer into an HPLC vial for analysis.

The recommended dilution procedure is given in the following table.

Sample type	Fortified Concentration (mg/kg)	Sample Weight (g)	Extract Volume (mL)	Dilution (mL-mL)	Dilution Factor
Reagent blank[1]	N/A	N/A	50	0.2-1	50
Control[2]	N/A	5	50	0.2-1	50
LOQ	0.05	5	50	0.2-1	50
10 × LOQ	0.5	5	50	0.04-1	250

N/A = Not Applicable.
[1] Use acetonitrile for the reagent blank.
[2] Dilute additional control sample extracts for matrix matched standards if required.

LC-MS/MS CONDITIONS

HPLC Parameters:

Instrument:	Shimadzu Nexera series HPLC system
Column#:	Phenomenex Kinetex 2.6 µm phenyl-hexyl 3 × 50 mm
Mobile Phase A#:	0.1% Formic acid in water
Mobile Phase B#:	0.1% Formic acid in acetonitrile
Flow Rate:	0.5 mL/min

Gradient:

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	80	20
0.5	80	20
3.0	0	100
4.0	0	100
4.1	80	20
5.5	80	20

Run Time:	5.5 minutes
Column Temperature:	40°C
Autosampler Temperature:	4°C
Injection Volume:	50 µL
Retention Time:	Approx. 2.5 minutes

Valco Valve Diverter:

Time (min)	Position
0	A (to waste)
0.5	B (to MS)
5.5	A (to waste)

MS/MS Parameters:

Instrument:	AB Sciex API 5000 Triple Quadrupole Mass Spectrometer
Ionisation Type#:	Electrospray (ESI)
Polarity#:	Positive
Scan Type#:	Multiple reaction monitoring (MRM)
Ion Spray Voltage:	4500 V
Collision Gas (CAD):	8
Curtain Gas (CUR):	20
Gas Flow 1 (GS1):	40
Gas Flow 2 (GS2):	40
Vaporiser Temperature (TEM):	550°C
Interface Heater (ihe):	On
Entrance Potential (EP):	10
Declustering Potential (DP):	50
Collision Exit Potential (CXP)	25
Resolution Q1/Q3:	Unit/Unit

Transition Name:	MRM Transition Ions Monitored	Collision Energy (CE)	Dwell Time (ms)
Dicloran (Primary):	207/190	23	200
Dicloran (Confirmatory):	207/160	35	200

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

All peak measurements and calculations are performed on Analyst 1.6.2. From the measured peak area, where the calibration fit is linear as in this study, Analyst uses the following formula to calculate the concentration of test substance present in the sample extract.

$$x = \frac{(y - c)}{m} \times DF$$

Where:-

x = concentration of test substance in sample (µg/kg)
y = area of peak due to test substance
m = gradient
c = Y intercept on calibration graph
DF = sample dilution factor

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

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

Where:-

A = concentration found in fortified sample (µg/kg)
S = concentration added to fortified sample (µg/kg)

METHOD CRITERIA

For the analysis by LC-MS/MS to be considered successful the following criteria should be met.

- At least 5 calibration standards will be used in the determination of the calibration line.
- The correlation coefficient (r) for the calibration line will be ≥ 0.995 with a 1/x weighting.
- All sample extracts should be within the appropriate range of calibration standards.
- Mean recovery from fortified samples should be within the range of 70 to 110% at each concentration.
- Precision of fortified sample recoveries should be ≤ 20% RSD at each concentration.
- The control sample should not contain interference > 30% of the LOQ at the retention time of the test substance.

GENERAL HANDLING CONTROL CATEGORIES

CATEGORY Main Division	CONTROL Name and Specification
1	GLOVES
a	Disposable latex
b	Disposable nitrile
c	Rubber gloves
d	Specific type for the job (see assessment giving details)
2	PROTECTIVE CLOTHING
a	Laboratory coat or equivalent
b	Disposable overalls
c	Oversleeves
d	Overshoes
e	Plastic apron
3	EYE/FACE PROTECTION
a	Safety glasses to BS 2092/2 C or better
b	Face shield to BS 2092/2 C or better
c	Safety goggles to BS 2092/2 C or better
4	ENGINEERING CONTROLS
a	Open bench in ventilated area
b	Fume cupboard to BS 7258
c	Laminar flow cabinet to BS 5295 Class 1
d	Re-circulating fume chamber
e	Radioisotope lab
f	Biohazard lab
g	Glove box
5	RESPIRATORY PROTECTIVE EQUIPMENT
a	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)