Summary

TRIBUFOS: Independent Laboratory Validation of Methodology for the Determination of Residues of Tribufos in Soil (Sandy Loam and Clay Loam) and Sediment (Sandy Silt Loam)

The objective of this study was to perform an independent laboratory validation for the determination of Tribufos in soil and sediment. The methodology to be validated is as described in Section 3 (Experimental Procedure) in the following document:

> 'Environmental analysis; BDG0137; Final Analytical Phase Report 23 March 2012, Stephen Brewin'

Samples were extracted by addition of acetone / hexane 95/5 followed by vigorous shaking. Extracts were evaporated to dryness and reconstituted in acetonitrile prior to analysis by LC-MS/MS.

LC-MS/MS instrumentation conditions were developed in conjunction with those detailed in the methodology in order to obtain sufficient response, linearity and specificity, using the different manufacturer of LC-MS/MS equipment held by the independent laboratory. The quantification and confirmation ion transitions were 315>169 and 315>57 respectively. The limit of detection (LOD) of the analytical system was determined as 0.025 ng/mL, equivalent to 0.0025 mg/kg in soil and sediment.

The limit of quantitation (LOQ) of the analytical method was confirmed as 0.01 mg/kg.

The chromatographic response of the analytes to the LC-MS/MS instrument was shown to be linear over the range of concentrations 0.025 to 2 ng/mL.

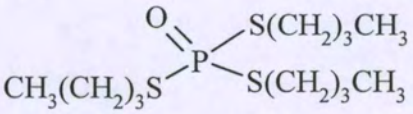
No significant interferences were found when the method was applied to control samples and analysed using solvent instrument calibration solutions, thus assuring the specificity of the method.

The method was validated at 0.01 and 0.1 mg/kg which is consistent with the original method. For each matrix type the mean recoveries for each fortification level and overall were within the acceptable range of 70 to 120% (see summary table) when analysed using solvent instrument calibration solutions, demonstrating accuracy (recovery) of the method. For each matrix type the relative standard deviation (RSD) obtained at each fortification level and overall was within the acceptable range of $\leq$ 20% when analysed using solvent instrument calibration solutions, demonstrating precision (repeatability) of the method.

2. Materials

2.1 Analytical standards

2.1.1 Tribufos technical

Chemical name (IUPAC)	S,S,S-tributyl phosphorothioate
Structure	
Storage conditions	Ambient
Batch number	NX81AX0015
CAS number	78-48-8
Purity	98.2%
Supplier	Sponsor
Re-test date	September 2012

Certificate of Analysis is presented in Appendix 1.

2.2 Untreated samples

Untreated soil and sediment was obtained by the Department of Bioanalysis, Huntingdon Life Sciences for use in this study. Untreated samples were stored at approximately +21°C (soil) or +4°C (Sediment) prior to use.

	Soil 1	Soil 2	Sediment
Source	Evesham	Bromsgrove	Calwich abbey
Textural class	Clay loam	Sandy loam	Sandy silt loam
pH	7.9	6.2	7.9
%Organic carbon	2.6	1.3	4.4
%Sand (USDA)	41	70	25
%Silt (USDA)	28	17	61
% Clay (USDA)	31	13	14

2.3 Reagents

A list of all reagents used is presented below:

Materials	Grade (or equivalent)
Acetonitrile	LC-MS/MS grade
Ammonium formate	Analytical reagent
Methanol	HPLC grade
Formic acid	Analytical reagent
Acetone	Glass distilled
Hexane	Analytical reagent
Octanol	Analytical reagent
Water	Ultra high purity (UHP)

2.4 Computer Systems

The computer systems with version number used on this study are as follows:

Applied Biosystems/MDS Sciex Analyst (version 1.4.2 or later) to acquire and quantify data

Xybion Pristima (version 6.2) for Pharmacy test item management

3. Experimental procedures

3.1 Modifications to the supplied method

Minor modifications were made to the LC-MS/MS instrument parameters in order to optimise response and specificity on the instrumentation used in the analytical laboratory; the LC-MS/MS instrument parameters used are detailed in Section 3.7.

3.2 Preparation of analytical standard solutions

3.2.1 Stock and fortification standard solutions

A weighed amount (corrected for purity if required) of the analytical standard was dissolved in acetonitrile to produce an individual stock standard solutions (1 mg/mLl). An aliquot of the stock standard solution was progressively diluted to 100 ng/mL and 10 ng/mL with acetonitrile to give fortification standard solutions.

3.2.2 Solvent-based instrument calibration solutions

The stock solution (1mg/L) was progressively diluted with acetonitrile to produce a series of instrument calibration solutions in the range 0.025 to 2 ng/mL. Aliquots of each calibration solution were injected to produce a standard curve.

3.3 Apparatus, glassware etc

Balances (various ranges)
Volumetric flasks (various sizes)
Syringes (various sizes)
Volumetric pipettes (various sizes)
Polyethylene pots (250 mL)
Ultra-Turrax T25 sample homogeniser
Polypropylene tubes (15 mL)
Pipettes (various sizes)

3.4 Preparation of reagents

Acetone:hexane (5:95 v:v)

Acetone (50 mL) is mixed thoroughly with Hexane (950 mL).

Water:methanol:formic acid (90:10:0.1 v:v:v) containing 0.01M ammonium formate

Methanol (100 ml), ammonium formate (0.6 g) and formic acid (1 ml) is added to HPLC water (900 ml) and mixed thoroughly prior to use.

Methanol:formic acid (100:0.1 v:v)

Methanol (1000 mL) is mixed thoroughly with formic acid (1 mL).

3.5 Validation

Sub-samples (10 g) of each untreated soil and sediment were fortified at known concentrations of the analytes, using either the 100 ng/mL or 10 ng/mL tribufos solutions and analysed according to the following regime:

2 untreated sub samples
5 untreated sub samples fortified at the LOQ (0.01 mg/kg wet weight)
5 untreated sub samples fortified at 0.1 mg/kg wet weight.

These samples were then processed using the analytical methodology described in Section 3.6. Each soil or sediment type was assayed in a separate analytical run against a separate calibration curve produced from the same calibration solutions.

3.6 Sample extraction procedure

1. Transfer a 10 g sub-sample of soil/sediment to a 100 mL polyethylene bottle.
2. Add fortification solution at this stage if required.
3. Add anhydrous sodium sulphate (approximately 5 g) and acetone:hexane (5:95 v:v, 45 mL) and securely cap the bottle.
4. Shake vigorously by hand for 10 seconds to break up the sediment.
5. Place on a mechanical shaker and shake at approximately 200 rpm for approximately 30 minutes.
6. Centrifuge at approximately 3500 rpm for approximately 3 minutes, to separate the phases.
7. Decant the supernatant through a funnel containing a Whatman Number 1 filter paper into a new 100 mL polyethylene bottle.
8. Repeat the extraction as steps 3 to 7, combining the extracts in the 100 mL bottle.
9. Dilute the extract to volume (100 mL) with acetone:hexane (5:95 v:v) and mix well.
10. Transfer an aliquot (1 mL) of the extract to a 15 mL polypropylene tube.
11. Add octanol (1 drop).
12. Evaporate to near dryness under nitrogen at approximately 30 - 40°C.
13. Reconstitute the sample extract in acetonitrile (10 mL), using ultrasonication to aid dissolution. Final matrix concentration ≡ 0.01 g soil/sediment / mL final extract.

3.7 LC-MS/MS analysis

Instrument:	Sciex API 4000
Data management system:	Analyst 1.4.2
Ionisation mode:	Positive Ionspray
Ion monitoring details:	*m/z* 315>169 *m/z* 315>57 (confirmatory)
Column:	Acquity UPLC® BEH C_{18} (2.1 mm x 50 mm, 1.7 µm), or equivalent
Column temperature:	45°C
Mobile phase A:	Water:methanol (90:10 v:v) + 0.01M ammonium formate + 0.1% formic acid
Mobile phase B:	Methanol:formic acid (100:0.1 v:v)

Gradient:

Time	%A	%B
0	30	70
0.2	30	70
2.0	5	95
2.5	5	95
3	30	70
4	30	70

Cycle time:	4 min
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Retention time:	approximately 1.9 minutes
LOQ:	0.01 mg/kg
LOD:	0.025 ng/mL (≡ 0.0025 mg/kg in sample matrix)

4. Calculation of results

Validation samples were quantified using the following equation:

$$\text{Residue found (mg/kg)} = x \times \frac{1}{M} \times D$$

Where x (residue concentration in final solution) was calculated using the linear regression

$$y = m\,x + c \qquad \text{where } x \text{ (concentration in ng/mL)} = \frac{y - c}{m}$$

c	=	intercept
m	=	slope
y	=	peak area of sample
M	=	matrix concentration (mL/mL)
D	=	dilution factor

Example calculation of Tribufos detected in sediment (sandy silt loam) fortified at 0.1 mg/kg (analytical identification ILV_0.1_4, analysis batch ILV04).

Linear regression $y = m\,x + c$

$$120615 = 137000x + 2110$$

where

$y = 120615$
m = 137000
c = 2110

$$\text{Therefore, concentration of Tribufos } (x) = \frac{120615 - 2110}{137000} = 0.863 \text{ ng/mL}$$

Matrix concentration = 0.01 g matrix/mL final extract
Dilution factor = 1

$$\text{Tribufos detected (mg/kg)} = \frac{0.863 \text{ ng/mL} \times 1}{0.01 g/\text{mL}} = 86.3 \text{ ng/mL} = 0.0863 \text{ mg/kg}$$

$$\text{Recovery (\%)} = \frac{0.086 \text{ mg/kg} \times 100}{0.1 \text{ mg/kg}} = 86\%$$