Summary

TRIBUFOS: Independent Laboratory Validation of Methodology for the Determination of Residues of Tribufos in Water (Surface and Ground Water)

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

> 'Environmental analysis; BDG0129; Final Analytical Phase Report 23 March 2012, Stephen Brewin'

Sample cleanup was by solid phase extraction on HLB cartridges.

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 0.0125 µg/L in Water.

The limit of quantitation (LOQ) of the analytical method was confirmed as 0.1 µg/L.

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.

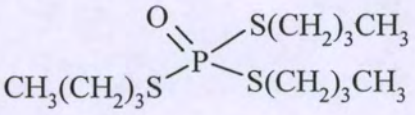
No significant interferences were found when the method was applied to control samples and analysed using solvent instrument calibration solutions, thus assuring the specificity of the method.

The method was validated at 0.1 and 1 µg/L which is consistent with the original method. For each matrix type the mean recoveries for each fortification level and the overall mean 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 the overall RSD 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

The certificate of Analysis is presented in Appendix 1.

2.2 Untreated samples

Untreated Water was obtained by the Department of Bioanalysis, Huntingdon Life Sciences for use in this study. Untreated samples were stored at approximately +4°C prior to use.

Source	Water Type	Properties
Anglian Water Denton Lodge Borehole 1	Ground water	Not available
Calwich Abbey Surface Water	Surface Water	Hardness as $CaCO_3$ = 121 mg/L Total Organic carbon = 6.4 mg/L Calcium = 42.8 mg/L Phosphorus = <0.1 mg/L Magnesium = 3.38 mg/L Suspended soils = 35 mg/L Nitrogen = 2.4 mg/L pH = 7.52

2.3 Reagents

A list of all reagents used is presented below:

Materials	Grade
Acetonitrile	LC-MS/MS grade
Ammonium formate	Analytical Reagent
Methanol	LC-MS/MS grade
Formic acid	LC-MS/MS grade
Water	Ultra high purity (UHP)

2.4 Computer Systems

The computer system 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

The sample volume taken through the extraction procedure was doubled to 10 mL and the volume of acetonitrile added prior to SPE step was increased (from 2 mL/ 5 mL of sample to 2.5 mL/ 5 mL of sample) in order to achieve acceptable recovery at the low fortification level.

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 a stock standard solution (1mg/mL). 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 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 standard solution were injected to produce a calibration curve.

3.3 Apparatus, glassware etc

Balances (various ranges)
Volumetric flasks (various sizes)
Volumetric pipettes (various sizes)
Polypropylene tubes (15 mL)
Pipettes (various sizes)

3.4 Preparation of reagents

Acetonitrile:water (30:70 v:v)

acetonitrile (30 mL) is mixed thoroughly with water (70 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 mL) of each untreated water were fortified at known concentrations of the analytes, by addition of 100 µL of either the 100 ng/ml or 10 ng/ml Tribufos solutions and the sample mixed and analysed according to the following regime:

2 untreated sub samples
5 untreated sub samples fortified at the LOQ (0.1 µg/L)
5 untreated sub samples fortified at 1 µg/L

These samples were then processed using the analytical methodology described in Section 3.6. Ground water and surface water samples were assayed in the same analytical run against the same calibration curve.

3.6 Sample extraction procedure

1. Transfer an aliquot of sample water (10 mL) to a 15 mL polypropylene tube.
2. Add fortification solution at this stage if required.
3. Add an aliquot (5 mL) of acetonitrile and mix well.
4. Condition the Oasis HLB SPE cartridge with acetonitrile (3 mL) and water (3 mL), discarding the eluate.
5. Load the extract from step 3 onto the SPE cartridge, discarding the eluate.
6. Wash the cartridge with an aliquot (3 mL) of acetonitrile:water (30:70 v:v), discarding the eluate, allowing the cartridge to have air pumped through for approximately 30 seconds to remove excess solvent.
7. Elute the SPE cartridge with an aliquot (4.5 mL) of acetonitrile, collecting in a 15 mL polypropylene tube.
8. Dilute the final extract to volume (10 mL) with acetonitrile. Final matrix concentration = 1 mL sample water / mL final extract.
9. Perform any further dilutions using acetonitrile, as required.
10. Quantify the samples by the use of LC-MS/MS.

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
Sample temperature:	+4°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 2 minutes
LOQ:	0.1 µg/L
LOD:	0.025 ng/mL (= 0.0125 µg/L in sample matrix)

4. Calculation of results

Validation samples were quantified using the following equation:

$$\text{Residue found } (\mu g/L) = x \times \frac{1}{M} \times D$$

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 surface water soil fortified at 1 µg/L.

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

$$112570 = 155000x + 672$$

where
$y = 112570$
$m = 155000$
$c = 672$

$$\text{Therefore, concentration of Tribufos } (x) = \frac{112570 - 672}{155000} = 0.722 \text{ ng/mL}$$

Matrix concentration = 2 mL matrix/mL final extract
Dilution factor = 2

$$\text{Tribufos detected } (\mu g/L) = \frac{0.722 \text{ ng/mL} \times 2}{2 \text{ mL/mL}} = 0.722 \text{ ng/mL} = 0.722 \text{ µg/L}$$

$$\text{Recovery } (\%) = \frac{0.722 \text{ µg/L} \times 100}{1 \text{ µg/L}} = 72\%$$