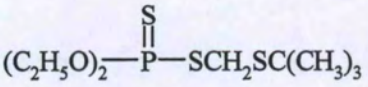
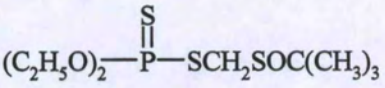
2. Materials

2.1 Analytical standards

2.1.1 Terbufos

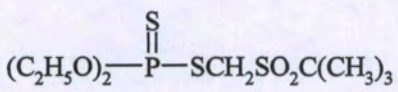
Identity	Terbufos (also known as Terbufos Technical)
Chemical name (IUPAC)	S-tert-butylthiomethyl O,O-diethyl phosphorodithioate
Structure	
Storage conditions	Ambient
Batch number	0351H01HC
CAS number	13071-79-9
Purity	89.3%
Supplier	Sponsor
Re-test date	17 December 2012

2.1.2 Terbufos Sulfoxide

Identity	Terbufos sulfoxide
Chemical name (IUPAC)	S-[(tert-butylsulfinyl)methyl] O,O-diethyl phosphorodithioate
Structure	
Storage conditions	Refrigerator (approx +4°C)
Batch number	AC11957-97B
CAS number	10548-10-4
Purity	95.8%

Supplier	Sponsor
Expiry date	01 January 2013

2.1.3 Terbufos Sulfone

Identity	Terbufos sulfone
Chemical name (IUPAC)	S-[(tert-butylsulfonyl)methyl] O,O-diethyl phosphorodithioate
Structure	
Storage conditions	Frozen (approx -20°C)
Batch number	L67-206
CAS number	56070-16-7
Purity	99.5%
Supplier	AMVAC Chemical Corporation
Re-test date	01 April 2017

The Certificates of Analysis are 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, Sudbury Borehole	Ground water	Not available
Calwich Abbey Surface Water	Surface Water	Hardness as $CaCO_3$ = 217 mg/L Total Organic carbon = 4.5 mg/L Calcium = 74.3 mg/L Phosphorus = 0.1 mg/L Magnesium = 7.69 mg/L Suspended soils = 39 mg/L Nitrogen = 3.7 mg/L pH = 7.66

2.3 Reagents

A list of all reagents used is presented below:

Materials	**Grade**
Anglian Water , Sudbury Borehole	Ground water
Calwich Abbey surface water	Surface water
Acetonitrile	LC-MS grade
Ammonium formate	LC-MS grade
Methanol	LC-MS grade
Formic acid	LC-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

After two validation attempts where low recoveries were obtained for the terbufos analyte (terbufos sulfoxide and terbufos sulfone recoveries were good in every batch analysed) in both types of water, the original validation laboratory was contacted with the Sponsor's permission to discuss possible reasons for this.

Following the discussions, a set of experiments were agreed to investigate the issue (these were; a) effect of increasing volume of elution solvent used, b) using an alternative dilution solvent and c) use of alternative elution solvents). Acceptable improvement in terbufos recovery was observed with option c), using an elution solvent of 50:50 v/v of acetonitrile /methanol. A validation batch was prepared in both types of water using this modification, but the improved recovery was not replicated.

A literature search was conducted of the published literature on terbufos and its analysis. It was found that there had been some observations of terbufos instability (mainly in water). After a discussion of various options for alternative modifications with the sponsor, it was agreed to investigate the effect of conducting the analysis with the water samples chilled on ice (as this was a minimal modification). This investigation did show improved and acceptable recovery of terbufos, without any other modifications to the original method. The validation batches were then performed using this minor modification, which were successful with acceptable recoveries of all three analytes.

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 standards were dissolved in acetonitrile to produce individual stock standard solutions of terbufos, terbufos sulfoxide and terbufos sulfone. An aliquot of each stock standard solution was taken to prepare a mixed secondary solution. The mixed secondary solution was progressively diluted to, 1 µg/mL and 100 ng/mL with acetonitrile to give fortification standard solutions.

3.2.2 Solvent-based instrument calibration solutions

The 1 µg/mL fortification solution was progressively diluted with acetonitrile:water (60:40 v/v) to produce a series of instrument calibration solutions in the range 0.1 to 10 ng/mL.

3.3 Apparatus, glassware etc

Balances (various ranges)
Volumetric flasks (various sizes)
Polypropylene tubes (15 mL)
Automatic pipettes (various sizes)
Oasis HLB SPE cartridge (60 mg, 3 mL)
Measuring cylinders (various sizes)

3.4 Preparation of reagents

Acetonitrile:water (60:40 v:v)

acetonitrile (60 mL) is mixed thoroughly with water (40 mL).

Acetonitrile:water (20:80 v:v)

acetonitrile (20 mL) is mixed thoroughly with water (80 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 of each type of untreated water were fortified at known concentrations of the analytes (using mixed fortification solutions containing all three analytes), 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.

3.6 Sample extraction procedure

1. Transfer an aliquot of sample water (25 mL) to a 50 mL polypropylene tube.
2. Add fortification solution at this stage if required.
3. Add an aliquot (10 mL) of methanol 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 (5 mL) of acetonitrile:water (20:80 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 (3 mL) of acetonitrile, collecting in a 15 mL polypropylene tube.
8. Dilute the final extract to volume (5 mL) with water. Final matrix concentration ≡ 5 mL sample water / mL final extract.
9. Perform any further dilutions using acetonitrile: water (60:40 v:v), as required.
10. Quantify the samples by the use of LC-MS/MS.

Note: Water samples are kept on ice until loaded onto the SPE cartridge for extraction (i.e. during steps 1 to 4). Batches must extracted without interruption.

3.7 LC-MS/MS analysis

Instrument:	Sciex API 4000	
Data management system:	Analyst 1.4.2	
Ionisation mode:	Positive Ionspray	
Ion monitoring details:	Terbufos:	*m/z* 289>103
		m/z 289>233 (confirmatory)
	Terbufos sulfoxide:	*m/z* 305>187
		m/z 305>243 (confirmatory)
	Terbufos sulfone:	*m/z* 321>171
		m/z 321>265 (confirmatory)
Column:	Acquity UPLC® BEH C_{18} (2.1 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	50	50
0.2	50	50
2.0	5	95
2.5	5	95
3	50	50
4	50	50

Cycle time: 4 min

Injection volume: 10 µL

Flow rate: 0.5 mL/min

Retention time: Terbufos: approx. 2.2 minutes
Terbufos sulfoxide: approx. 1.3 minutes
Terbufos sulfone: approx. 1.3 minutes

LOQ: 0.1 µg/L

LOD: 0.1 ng/mL (≡ 0.02 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 terbufos detected in surface water soil fortified at 1 µg/L (analytical batch BDG0131/ILV10).

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

$$84003 = 20700x + 94.9$$

where $y = 84003$
$m = 20700$
$c = 94.9$

$$\text{Therefore, concentration of terbufos } (x) = \frac{84003 - 94.9}{20700} = 4.05353 \text{ ng/mL}$$

Matrix concentration = 5 mL matrix/mL final extract
Dilution factor = 1

$$\text{Terbufos detected } (\mu g/L) = \frac{4.05353 \text{ ng/mL} \times 1}{5 \text{ mL/mL}} = 0.811 \text{ ng/mL} = 0.811 \text{ µg/L}$$

$$\text{Recovery } (\%) = \frac{0.811 \text{ µg/L} \times 100}{1 \text{ µg/L}} = 81.1\%$$