2. Materials

2.1 Analytical standards

2.1.1 Naled technical

Chemical name	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Source	Sigma
Storage conditions	Refrigerated (recommended 2-8°C)
Batch number	SZB8092XV
Purity	96.6%
Re-test date	01 April 2014

2.1.2 Dichlorvos

Identity	2,2-dichlorovinyl dimethyl phosphate
Source	Sigma
Storage conditions	-20°C
Batch number	SZBA223XV
Purity	98.9%
Re-test date	11 August 2013

Certificates of Analysis are presented in Appendix 1.

2.2 Untreated samples

Materials	**Grade (or equivalent)**	**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

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

2.3 Reagents

A list of all reagents used is presented below:

Materials	Grade (or equivalent)
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 was Applied Biosystems/MDS Sciex Analyst (version 1.4.2 or later) to acquire and quantify the data.

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

Weighed amounts (corrected for purity if required) of the analytical standards were dissolved in acetonitrile to produce individual stock standard solutions (1 mg/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 standard solution was progressively diluted with methanol to produce a series of instrument calibration solutions in the range 0.05 to 2.5 ng/mL.

3.3 Apparatus, glassware etc

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

3.4 Preparation of reagents

Methanol:water (10:90 v:v)

Methanol (100 mL) is mixed thoroughly with water (900 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, with mixed solutions of the 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 (10 mL) to a 15 mL polypropylene tube.
2. Add fortification solution at this stage if required.
3. Add an aliquot (1 mL) of methanol and mix well.
4. Condition the Oasis HLB SPE cartridge with methanol (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 methanol:water (10:90 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 methanol, collecting in a 15 mL polypropylene tube.
8. Dilute the final extract to volume (5 mL) with methanol. Final matrix concentration ≡ 2 mL sample water / mL final extract.
9. Perform any further dilutions using methanol, as required.
10. Quantify the samples by the use of LC-MS/MS.

3.7 LC-MS/MS analysis

Instrument:	AB Sciex API 4000
Mode:	Ionspray positive
Ion monitoring details:	Naled: *m/z* 381>127 Naled: *m/z* 383>127 (confirmatory) Dichlorvos: *m/z* 221>127 Dichlorvos: *m/z* 223>127 (confirmatory)
Column:	Acquity UPLC® BEH C_{18} (2.1 mm x 50 mm, 1.7 µm)
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	80	20
0.2	80	20
2.0	5	95
2.5	5	95
3	80	20
4	80	20

Cycle time:	4 min
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Retention times:	Naled: approximately 1.9 minutes Dichlorvos: approximately 1.6 minutes
LOQ:	0.1 µg/L
LOD:	0.05 ng/mL (≡ 0.025 µg/L in sample matrix)

4. Calculation of results

Test 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 Naled detected in surface water fortified at 1 µg/L

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

$$42197 = 20900x + 61.4$$

where
$y = 42197$
$m = 20900$
$c = 61.4$

$$\text{Therefore, concentration of Naled } (x) = \frac{42197 - 61.4}{20900} = 2.02 \text{ ng/mL}$$

Matrix concentration = 2 mL matrix/mL final extract
Dilution factor = 1

$$\text{Naled detected } (\mu g/L) = \frac{2.02 \text{ ng/mL} \times 1}{2 \text{ mL/mL}} = 1.01 \text{ ng/mL} = 1.01 \text{ µg/L}$$

$$\text{Recovery } (\%) = \frac{1.01 \,\mu g/L \times 100}{1.0 \,\mu g/L} = 101\%$$