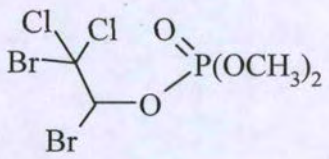
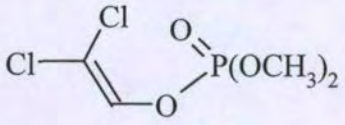
2. Materials

2.1 Analytical standard – naled

Identity	Naled technical
Other name	Dibrom
Chemical name (IUPAC)	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Structure	
Storage conditions	Refrigerated (recommended 2-8°C)
Batch number	SZB8092XV
CAS number	300-76-5
Purity	96.6%
Supplier	Sigma-Aldrich
Re-test date	1 April 2014

2.2 Analytical standard – dichlorvos

Identity	Dichlorvos
Other name	DDVP
Chemical name (IUPAC)	2,2-dichlorovinyl dimethyl phosphate
Structure	
Storage conditions	Dry, at room temperature, under nitrogen
Batch number	VP-164
CAS number	62-73-7
Purity	99.1%
Supplier	Sponsor
Re-test date	November 2013

Certificates of Analysis are presented in Appendix 1.

2.3 Control matrices

The ground water was obtained from Anglian Water Denton Lodge Borehole 1 and the surface water was obtained from Diss Mere. Upon receipt the water samples were allocated a unique Huntingdon Life Sciences, Environmental Analysis Department identification number. The water was characterised in separate studies and the characterisation data is presented in the following table:

Parameter	Found value (surface water)	Found value (ground water)
pH	8.07	7.62
Dissolved Oxygen (analysed on the date of sampling	7.32 mgO_2/L	10.57 mgO_2/L
Conductivity	376 µS/cm	397 µS/cm
Alkalinity	198 mg/l as $CaCO_3$	568 mg/l as $CaCO_3$
Total Hardness	221 mg/l as $CaCO_3$	228 mg/l as $CaCO_3$
Total Organic Carbon	16.198 mgC/L	1.8 mgC/L
Dissolved Organic Carbon	13.814 mgC/L	3.1 mgC/L

3. Methods

3.1 Validation

Sub-samples of each of the two water types were fortified with known concentrations of both of the analytes simultaneously and analysed according to the following regime:

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

These samples were then analysed using the analytical methodology, with each sample injected onto the chromatographic system once.

3.2 Final extract stability

An experiment was set up to demonstrate the stability of the analytes under the typical storage conditions of the final extracts if they are not quantified immediately after preparation. Processed control extracts, fortified with naled and dichlorvos were stored at approximately -20°C in the dark (i.e. in a freezer).

Aliquots of each of the control sample extracts (prepared in methanol) were fortified with naled and dichlorvos at a concentration of 2 ng analyte/mL of final extract. The concentration of analytes in the stored extracts was determined at day 0 and after 7 days. The concentration of the analytes in freshly fortified control extracts was also determined at that time.

3.3 Matrix effects

Any possible sample matrix effects were investigated by the comparison of the instrument response to the analytes in the fortified final extract samples with the response of the analytes in solvent based calibration standard solutions prepared at the same time.

3.4 Analytical method

Samples were extracted and cleaned up using solid phase extraction (SPE) cartridges. Quantitation was performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS), monitoring ion transitions *m/z* 381>127 and *m/z* 221>127 for naled and dichlorvos respectively. In addition, transitions *m/z* 383>127 and *m/z* 223>127 were also monitored for confirmation purposes, for each analyte respectively. Calculations were performed based on the peak areas of the resulting chromatographic peaks.

The analytical method used in the laboratory is presented in Appendix 3.

3.5 Fortification/calibration solutions

Individual stock standard solutions (1 mg/mL) of the two analytes were prepared by dissolving an accurately weighed amount in a suitable volume of acetonitrile, correcting for purity as appropriate. These stock solutions were further diluted with acetonitrile to produce mixed fortification solutions at 10 µg/mL, 1 µg/mL, 0.1 µg/mL and 0.01 µg/mL concentrations.

The instrument calibration solutions, over the concentration range 0.05 ng/mL to 2.5 ng/mL, were prepared by serial dilution of the fortification solution in methanol, as detailed below:

Standard solution used (ng/mL)	Volume taken (mL)	Final volume (mL)	Nominal concentration (ng/mL)
100	0.25	10	2.5
100	0.2	10	2
100	0.15	10	1.5
100	0.1	10	1
100	0.08	10	0.8
100	.0.04	10	0.4
1	2	10	0.2
1	1	10	0.1
1	0.5	10	0.05

3.6 Calculation of results for validation samples

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 0.1 µg/L (analytical identification 11/00/11826 F0.1 A, analysis batch 1). The primary data for this sample is presented in Table 14, Appendix 2.

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

$$7.16905e3 = 37665.8x - 125.157$$

where
$y = 7.16905e3$
$m = 37665.8$
$c = -125.157$

$$\text{Therefore, concentration of naled } (x) = \frac{7.16905e3 + 125.157}{37665.8} = 0.1937 \text{ ng/mL}$$

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

$$\text{Naled detected } (\mu g/L) = \frac{0.1937 \text{ ng/mL} \times 1}{2 \text{ mL/mL}} = 0.097 \text{ ng/mL} = 0.097 \text{ µg/L}$$

$$\text{Recovery } (\%) = \frac{0.097 \text{ µg/L} \times 100}{0.1 \mu g/L} = 97\%$$

Appendix 3 Analytical Method

DETERMINATION OF NALED AND DICHLORVOS IN WATER

1. General principle

Samples are extracted and cleaned up using solid phase extraction (SPE) cartridges. Quantitation is performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

2. Apparatus, glassware etc

Balances (various ranges)
Volumetric flasks (various sizes)
Syringes (various sizes)
Volumetric pipettes (various sizes)
Polypropylene tubes (15 mL)
Measuring cylinders (various sizes)
Solid phase extraction vacuum manifold

3. Materials	**Typical Grade (or equivalent)**
Acetonitrile	HPLC
Ammonium formate	AR
Methanol	HPLC
Formic acid	AR
Water	HPLC
Oasis HLB cartridges (60 mg, 3 mL)	

4. Preparation of reagents

Preparation of methanol:water (10:90 v:v) - methanol (100 mL) is mixed thoroughly with water (900 mL).

Preparation of 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.

Preparation of methanol:formic acid (100.0.1 v:v) - methanol (1000 mL) is mixed thoroughly with formic acid (1 mL).

Note: variable quantities of the above may be prepared by adjusting the constituent quantities accordingly.

5. Analytical standard solutions

An appropriate amount of the test substances (corrected for purity) are accurately weighed and dissolved in acetonitrile to give the individual stock standard solutions. Appropriate dilutions of the stock standard solutions are made with acetonitrile to give mixed fortification standard solutions.

The mixed fortification solutions are progressively diluted with methanol to produce a series of instrument calibration solutions in the range 0.05 to 2.5 ng/mL.

6. Procedure

6.1 Transfer an aliquot of sample water (10 mL) to a 15 mL polypropylene tube.
6.2 Add fortification solution at this stage if required.
6.3 Add an aliquot (1 mL) of methanol and mix well.
6.4 Condition the Oasis HLB SPE cartridge with methanol (3 mL) and water (3 mL), discarding the eluate.
6.5 Load the extract from step 6.3 onto the SPE cartridge, discarding the eluate.
6.6 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.
6.7 Elute the SPE cartridge with an aliquot (4.5 mL) of methanol, collecting in a 15 mL polypropylene tube.
6.8 Dilute the final extract to volume (5 mL) with methanol. Final matrix concentration ≡ 2 mL sample water / mL final extract.
6.9 Perform any further dilutions using methanol, as required.
6.10 Quantify the samples by the use of LC-MS/MS.

7. Flow chart of analytical procedure

Transfer 10 mL water sample to a 15 mL tube
↓
Fortify with analytical standard (if required)
↓
Add aliquot of methanol and mix well
↓
Extraction/cleanup by OASIS HLB SPE cartridge
↓
Dilute to volume with methanol
↓
Quantitative analysis by LC-MS/MS

8. LC-MS/MS conditions

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), 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	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 1.5 minutes Dichlorvos: approximately 1.3 minutes
LOQ:	0.1 µg/L
LOD:	0.05 ng/mL (≡ 0.025 µg/L in sample matrix)

NOTE – alternative instruments may also be used, operated under conditions that are considered to be equivalent to those described above. However, some differences may be observed in the resulting data, such as slight differences in analyte retention times, or the observed sensitivity of the ion transitions monitored.