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APPLICATION NOTE 10580

Methodology and validation results for analyzing pesticides in water by large volume PTV GC-MS/MS

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Keywords

PTV, TSQ 8000 EVO, CIP2, EU WFD, Pesticides, Water analysis

Goal

To develop and validate a method for analyzing pesticides in water by large volume PTV GC-MS/MS

Introduction

The European Union (EU) Water Framework Directive was introduced in August 2013, amending EU directive 2000/60/EC and 2008/105/EC and laying down a strategy against the pollution of water to be applied to all EU member states. This strategy involves the identification of priority substances and the monitoring of different classes of contaminants, such as several pesticide compounds.

EU member states have the flexibility to apply an Environmental Quality Standard (EQS) for an alternative matrix or, where relevant, an alternative biota taxon, for example sub-phylum Crustacean, paraphylum “fish”, class Cephalopodan or class Bivalvia (mussels and clams). The directive encourages the development of novel monitoring methods such as passive sampling and other tools.

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In the United Kingdom (UK) specifically, Chemical Investigation Programme 2 (CIP2) UK regulations investigates the occurrence, sources, and removal of trace substances in wastewater treatment facility effluent. This regulation helps to establish priorities for premeditative action to ensure surface waters meet new EQS.

The directive poses challenges to the water laboratories, mainly in terms of monitoring the various compounds at a very low required level. In many laboratories, the approach to reach these levels would require a capital equipment investment as well as a different approach to sampling. For instance, many liters of sample need to be taken for extraction. This application note describes a classic method of sample extraction with a standard volume of 1 liter, followed by large volume injection and MS/MS detection for optimal sensitivity.

The laboratories are following the guidelines and definitions as stated below in validating the methodologies. In this application note the results of these parameters are shown. The limit of quantitation (LOQ) is the level at which the analyte can be accurately quantified, while still achieving the required level of precision (typically 10–25%). As defined by the Directive, the LOQ is 2.15 times the limit of detection (LOD).

Generally speaking, the EQS is defined as being seven times the LOD. This value is used routinely as part of the in-house quality control and acquired with each batch of samples. It is also a very low-level spike, but the %RSD/recovery targets for most compounds are achieved.

The Directive values of LOD, LOQ, and EQS are all different for each compound. However, the laboratory has a single concentration spiking solution as this is far more practical to handle to prepare the solutions and to review data and results.

Experimental

The Environment Agency has the responsibility to develop methods that are both easily reproducible and affordable in the laboratories across the UK. The laboratory has carefully considered newer developments, such as passive sampling and automated sample preparation, and has chosen to develop a preparative method based on classic liquid/liquid extraction. This way both the quality of the recovery results and affordability is ensured.

Please note: The required EQS and the required detection limits in water for heptachlor and its epoxides in the legislation is at extremely low levels. The EQS for these three compounds is 0.0000002 µg/L or 0.2 fg/L. These limits of detection are so low that they would require an enormous amount of water to be handled for liquid/liquid extraction or an alternative solution like for instance passive sampling would need to be investigated (currently outside of the scope of this laboratory's requirements).

Sample preparation

It is important for laboratories to keep the sampling at a practical level to facilitate transport and general sample handling. The 1 L of water sample is extracted with 25 mL of dichloromethane and 5 mL of isohexane. The strong solvent offers efficient extraction of a wide range of pesticides with different polarities. The two solvents are taken off, combined, and dried with sodium sulphate, and subsequently concentrated down to 0.5 mL using a TurboVap® II system. Since the application is developed to perform large volume injections, it is critical to have a repeatable solvent mix before injecting onto a GC. The dichloromethane fraction is evaporated completely, using isohexane as a keeper. The end solvent is therefore 100% isohexane.

GC-MS experimental conditions

Compound separation and detection was achieved using a Thermo Scientific™ TRACE™ 1310 GC system coupled with a Thermo Scientific™ TSQ™ 8000 EVO mass spectrometer. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, injecting 60 µL using the enrichment feature on the Thermo Scientific™ Instant Connect-PTV module equipped with a Thermo Scientific™ baffled liner (P/N 453T2120) and a Thermo Scientific™ TraceGOLD™ TG-35MS column with the following dimensions: 30 m × 0.250 mm × 0.25 µm (P/N 26094-1420).

Additional details of instrument parameters are shown in Tables 1–3.

Table 1A. GC and injection conditions (Oven and Carrier Methods).

Oven Method	
Initial temperature:	40.0 °C
Initial hold time:	3.00 min
Number of ramps:	3
Ramp 1 rate:	25.0 °C/min
Ramp 1 final temperature:	180.0 °C
Ramp 1 hold time:	0.00 min
Ramp 2 rate:	5.0 °C/min
Ramp 2 final temperature:	260.0 °C
Ramp 2 hold time:	5.00 min
Ramp 3 rate:	100.0 °C/min
Ramp 3 final temperature:	300.0 °C
Ramp 3 hold time:	5.00 min
Carrier Method	
Carrier mode:	Constant pressure
Carrier pressure:	14.12 psi
Vacuum compensation:	On
Carrier gas saver enable:	On
Carrier gas saver flow:	10.0 mL/min
Carrier gas saver time:	20.00 min
Carrier gas:	He
GC Column	
Column	TraceGOLD 35MS (P/N 26094-1420) 5 m × 0.32 mm ID retention gap (P/N 260G496P)
Liner	Baffled Liner (P/N 453T2120)

Table 1B. GC and injection conditions (PTV-Front Method).

PTV-Front Method	
PTV Large Volume mode:	60 µL
Temperature:	40 °C
Split flow:	30.0 mL/min
Splitless time:	1.00 min
Purge flow:	40.0 mL/min
Constant septum purge:	On
Use evaporation phase:	Yes
Use cleaning phase:	Yes
Use ramped pressure:	Yes
Transfer temperature delay:	0.10 min
Post-cycle temperature:	Cool Down
Injection pressure:	14.50 psi
Injection time:	0.35 min
Injection flow:	30.0 mL/min
Evaporation pressure:	14.50 psi
Evaporation rate:	1.0 °C/s
Evaporation temperature:	55 °C
Evaporation time:	1.00 min
Evaporation flow:	40.0 mL/min
Transfer pressure:	75 psi
Transfer rate:	14.5 °C/s
Transfer temperature:	280 °C
Transfer time:	3.50 min
Cleaning rate:	14.5 °C/s
Cleaning temperature:	280 °C
Cleaning time:	15.00 min
Cleaning flow:	500.0 mL/min

Table 2. Mass spectrometer parameters.

MS transfer line temperature:	300 °C
Ion source temperature:	300 °C
Ionization mode:	EI
Detector gain:	2.50E+06
Emission current:	50 µA

Table 3. Target compound and internal standard list and transitions.

Name	Precursor Mass	Product Mass	Collision Energy (eV)	RT (min)	Width (s)	Name 2	Precursor Mass 2	Product Mass 2	Collision Energy 2 (eV)	RT 2 (min)	Width 2 (s)
135-Trichlorobenzene	179.9	109.0	24	7.14	30	Chlorthalonil	265.8	170.0	24	17.53	30
135-Trichlorobenzene	179.9	109.0	14	7.14	30	Chlorthalonil	265.8	133.0	36	17.53	30
HCBD-C13	228.8	193.9	14	7.88	30	Aldrin	262.7	192.9	32	17.82	30
HCBD-C13	230.8	195.9	14	7.88	30	Aldrin	262.7	191.0	30	17.82	30
HCBD	222.9	187.9	14	7.88	30	Terbutryn	241.1	68.0	6	18.07	30
HCBD	224.9	189.9	14	7.88	30	Terbutryn	241.1	170.1	12	18.07	30
124-TCB	180.0	109.0	24	8.01	30	Chlorpyrifos-Ethyl	196.7	107.0	36	18.42	30
124-TCB	180.0	144.9	14	8.01	30	Chlorpyrifos-Ethyl	196.7	168.9	12	18.42	30
123-TCB-D3	182.9	111.0	24	8.47	30	Fenitrothion	277.0	109.0	16	18.56	30
123-TCB-D3	182.9	148.0	14	8.47	30	Fenitrothion	277.0	260.0	6	18.56	30
123-TCB	179.9	108.9	28	8.49	30	Parathion-D10	301.1	83.0	24	18.78	30
123-TCB	179.9	144.9	16	8.49	30	Parathion-D10	301.1	115.1	10	18.78	30
Dichlorvos-D6	115.0	83.0	6	8.98	30	Isodrin	192.9	123.0	28	19.31	30
Dichlorvos-D6	191.0	99.1	12	8.98	30	Isodrin	192.9	157.2	20	19.31	30
Dichlorvos	185.0	93.0	12	9.02	30	p,p´-Dichlorobenzophenone	111.0	75.1	12	19.45	30
Dichlorvos	186.9	93.0	12	9.02	30	p,p´-Dichlorobenzophenone	139.0	111.0	12	19.45	30
Pentachlorobenzene	249.8	214.8	16	11.55	30	Pendimethalin-D5	255.2	164.1	10	19.56	30
Pentachlorobenzene	249.8	178.5	24	11.55	30	Pendimethalin-D5	255.2	193.1	5	19.56	30
Trifluralin-D14	267.0	163.1	12	11.91	30	Pendimethalin	252.1	162.0	8	19.64	30
Trifluralin-D14	315.1	267.1	8	11.91	30	Pendimethalin	252.1	191.3	8	19.64	30
Trifluralin	306.1	206.0	10	12.02	30	cis-Heptachlor-Epoxide	352.8	262.9	16	19.83	30
Trifluralin	306.1	264.1	8	12.02	30	cis-Heptachlor-Epoxide	262.9	192.9	30	19.83	30
Hexachlorobenzene	283.8	213.8	30	14.06	30	Trans-Heptachlor-Epoxide	352.9	253.0	16	20.12	30
Hexachlorobenzene	283.8	248.8	18	14.06	30	Trans-Heptachlor-Epoxide	183.0	155.0	12	20.12	30
HCH-Alpha-D6	221.9	148.0	18	14.23	30	Chlorfenvinphos	266.9	159.0	16	20.28	30
HCH-Alpha-D6	221.9	185.0	8	14.23	30	Chlorfenvinphos	266.9	203.0	10	20.28	30
HCH-Alpha	182.8	146.7	12	14.37	30	Irgarol 1051	253.2	182.1	12	20.51	30
HCH-Alpha	218.8	183.0	8	14.37	30	Irgarol 1051	182.0	109.1	10	20.51	30
Diazinon	137.1	84.1	12	14.75	30	Endosulfan-alpha	240.6	205.9	14	21.24	30
Diazinon	179.1	121.5	26	14.75	30	Endosulfan-alpha	194.7	125.0	22	21.24	30
Atrazine-D5	205.1	127.1	14	14.94	30	DDE-PP	246.0	176.1	28	21.96	30
Atrazine-D5	205.1	105.0	10	14.94	30	DDE-PP	317.8	246.0	20	21.96	30
Atrazine	200.0	132.0	8	15.00	30	Dieldrin	262.8	227.8	16	22.33	30
Atrazine	215.0	58.1	12	15.00	30	Dieldrin	262.8	190.9	30	22.33	30
Simazine	172.7	138.0	6	15.18	30	Endrin	280.8	245.3	8	23.53	30
Simazine	186.0	91.0	8	15.18	30	Endrin	262.8	192.9	30	23.53	30
HCH-Gamma	180.9	145.0	14	15.58	30	DDT-op	235.0	165.1	22	23.78	30
HCH-Gamma	180.9	109.0	26	15.58	30	DDT-op	235.0	199.5	10	23.78	30
Dimethoate-D6	149.1	114.0	10	15.66	30	TDE-PP	235.0	165.1	20	24.20	30
Dimethoate-D6	149.1	131.1	5	15.66	30	TDE-PP	235.0	199.0	14	24.20	30
Dimethoate	143.0	110.3	10	15.76	30	Endosulfan-beta	240.6	205.8	12	24.61	30
Dimethoate	143.0	111.0	10	15.76	30	Endosulfan-beta	158.9	123.0	12	24.61	30
HCH-Beta	180.9	145.0	14	16.45	30	DDT-D8	243.0	173.2	24	25.16	30
HCH-Beta	218.7	183.0	8	16.45	30	DDT-D8	245.1	173.1	22	25.16	30
Heptachlor	271.8	236.9	12	16.75	30	Aclonifen	264.0	194.1	14	25.19	30
Heptachlor	99.8	65.0	12	16.75	30	Aclonifen	264.0	212.1	12	25.19	30
Alachlor-D13	173.2	137.2	26	16.78	30	DDT-pp	235.0	165.1	22	25.28	30
Alachlor-D13	200.2	172.2	10	16.78	30	DDT-pp	235.0	199.5	10	25.28	30
Alachlor	188.1	130.0	32	16.98	30	Quinoxyfen	237.0	208.0	26	25.43	30
Alachlor	188.1	160.1	8	16.98	30	Quinoxyfen	307.0	237.0	18	25.43	30
HCH-Delta	218.8	182.9	8	17.40	30	Bifenox	172.9	137.9	16	29.85	30
HCH-Delta	218.8	146.5	20	17.40	30	Bifenox	341.1	281.0	12	29.85	30

Data processing

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software. The software allows linking the method through a database to both the instrument as well as the quantitation method. The ion ratio of the target compounds and their retention times are monitored, thus ensuring confident identification.

The Retention Time Alignment tool or RTA allows the user to keep the retention times of the method constant, even after column cutting or complete replacement. There is a substantial number of compounds in the methodology and the RTA allows the lab to run samples quickly after this type of maintenance.

Results and discussion

Calibration solution

The directive has very different EQS values for each compound, which makes it extremely difficult to build a comprehensive calibration curve. For practical reasons, the calibration curve is made using a stock solution with all the compounds in the same concentration.

Table 4 shows concentration of calibration solutions in 1 L of UHP water and calculated on column amount (assuming 100% extraction).

Table 4. Stock solution concentrations used to build the calibration curve.

Cal1	5000 pg/L	600 pg on column
Cal2	2000 pg/L	240 pg on column
Cal3	1000 pg/L	120 pg on column
Cal4	500 pg/L	60 pg on column
Cal5	200 pg/L	24 pg on column
Cal6	80 pg/L	9.6 pg on column
Cal7	40 pg/L	4.8 pg on column

Spiked river water to validate the method

1 L of surface water was spiked at different levels of concentration. Table 5 shows concentration levels and the calculated on-column amount .

Table 5. Surface water spikes.

80% of calibration range	4000 pg/L	480 pg on column
50% of calibration range	2500 pg/L	300 pg on column
5.6% of calibration range (EQS)	280 pg/L	33.6 pg on column
Limit of quantitation (LOQ)	80 pg/L	9.6 pg on column
Limit of detection (LOD)	40 pg/L	4.8 pg on column
Limit of detection (LOD)	20 pg/L	2.4 pg on column

Observations during method development

Choosing the most optimal transitions (selective and sensitive) is critical for sensitive analyte detection. At the very low target compound levels required, typically 80 pg/L, interferences from the matrix ions are likely. As an example, for dichlorvos, the normal choice for a precursor would be *m/z* 109. However, using this ion resulted in poor ion qualification at the sub 80 pg/L level. Switching to the less abundant and heavier precursor masses *m/z* 185 and *m/z* 187, although less abundant, resulted in better ion qualification due to better selectivity.

The focusing of the trichlorobenzenes at the front of the chromatogram proved difficult due to the 60 µL enriched injection volume. This was largely overcome by using constant pressure mode and a ramped pressure during the injection and transfer stage.

The maximum linear range for the application reached 5000 pg/L for all compounds in the scope of this methodology.

Validation results

River waters were spiked at the various levels described in Table 5. The samples were spiked and measured on 11 consecutive days to validate the scope of the analysis, assess data reproducibility, and to ascertain the limits of quantitation.

Figure 1 shows a series of spikes, focusing on g-HCH. This compound is a good performer with clear and defined peaks at 20 pg/L and with correct ion ratios from the lowest-level to the highest-level spike.

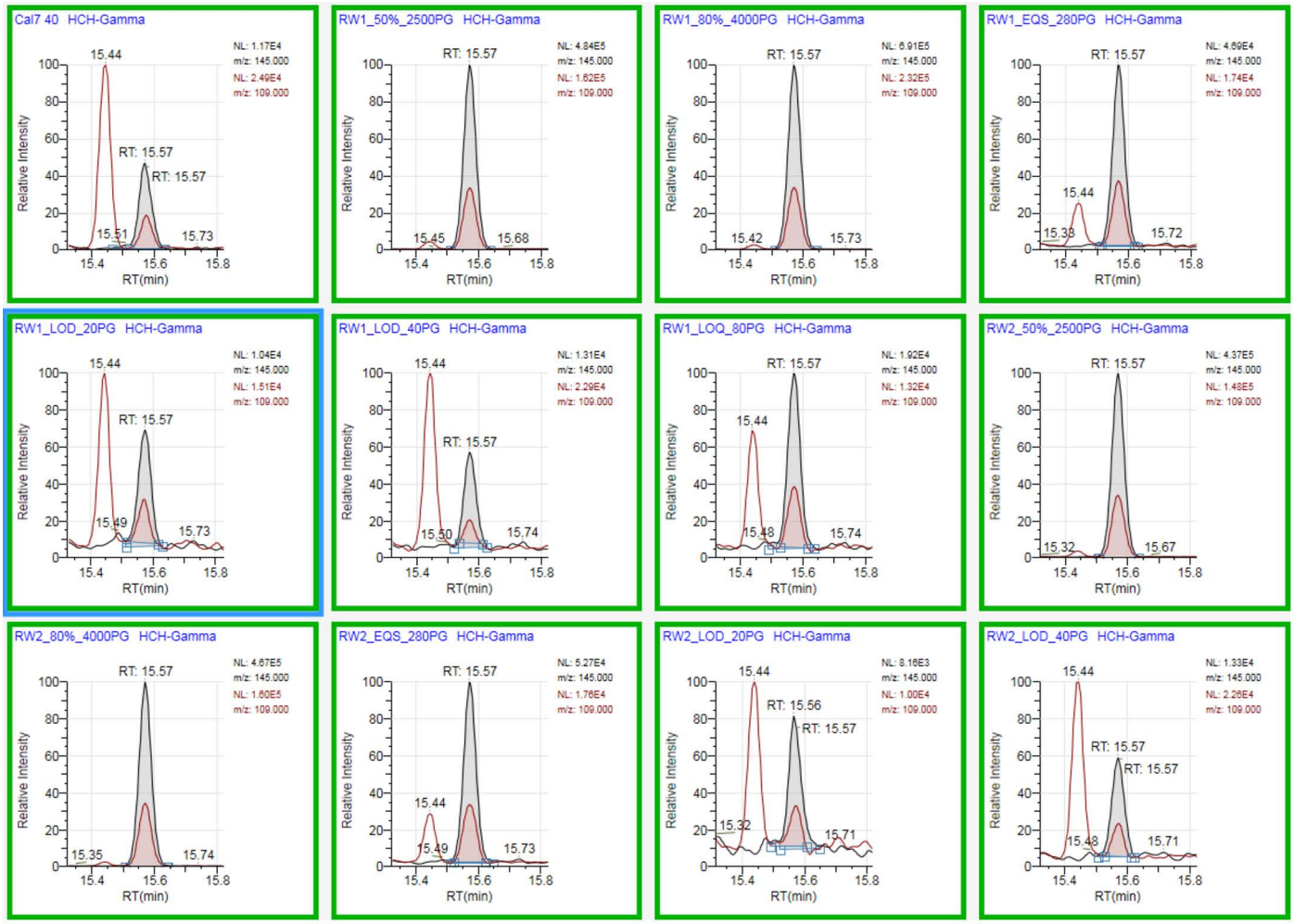

Figure 1. g-HCH in the lowest calibration level and the various river water spike levels (20 pg/L, 40 pg/L, 80 pg/L, 280 pg/L, 2500 pg/L, and 4000 pg/L) as listed in Table 5. The gray peak is the quantifying ion; the red peak is the confirming ion.

Additionally, the limit of quantitation and calibration curves are shown for dichlorvos, dicofol, and alpha-endosulfan (Figure 2). These compounds are highlighted based on the following:

- For dichlorvos: the precursor selected is not the most abundant, but the most selective in the matrix.
- For dicofol: this compound is very challenging to analyze because it is prone to break down during sample preparation as well as GC injection; the breakdown product is being analyzed i.e.: p,p´-dichlorobenzophenone.
- For alpha-endosulfan: this compound has a spectrum with many ions across the complete mass range and therefore any choice of ion is cumbersome and typically these ions have a relatively low response.

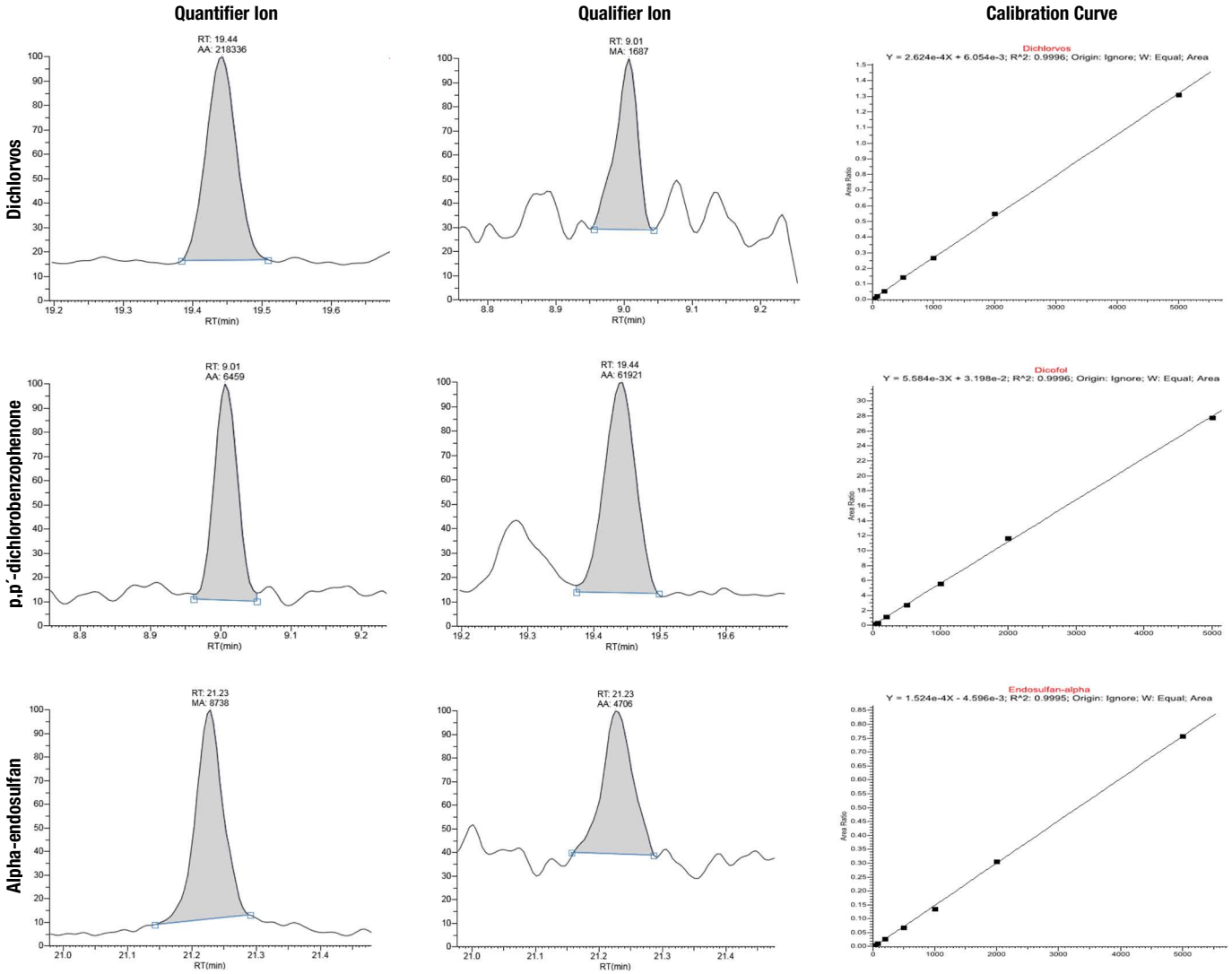

Figure 2. Dichlorvos, p,p´-dichlorobenzophenone, and alpha-endosulfan in river water spiked at 80 pg/L (the LOQ level), showing quantifier and qualifier ion and the calibration curves (across 40 to 5000 pg/L). Dichlorvos R^2= 0.9996; p,p´-dichlorobenzophenone R^2 = 0.9996; and endosulfan R^2 = 0.9995

Statistical evaluation over 11 batches

As part of the validation, 11 batches of calibration curves and spiked samples were analyzed. In these batches, all the spiked samples were extracted twice and analyzed in duplicate. Typical batch size is 23 samples. Over the 11 batches, the residual standard deviation target is 25%, and the recovery target is 100% ± 20% (Table 6). Due to background issues, chlorthalonil and pendimethalin were spiked at higher levels for the LOD, i.e. the EQS 280 pg/L spike was used for LOD purposes. This level is still within the requirement of the Directive. These two compounds are not in Table 6.

Table 6. Comparison of calibration methods, LODs, and LOQs of sodium thiosulfate.

11 Batches Results	% RSD at 80 pg/L in River Water	% Recovery at 80 pg/L in River Water	%RSD at EQS (280 pg/L) in River Water	% Recovery at EQS (280 pg/L) in River Water
1,2,4-Trichlorobenzene	23.0	103	6.4	94
1,3,5-Trichlorobenzene	12.0	104	17.5	101
Aclonifen	16.8	99	18.8	97
Alachlor	6.5	99	6.2	94
Aldrin	8.4	98	7.6	94
Atrazine	15.0	96	6.8	94
Bifenox	16.5	101	16.6	95
Chlorfenvinphos	17.0	97	23.3	94
Chlorpyrifos	24.9	108	13.3	95
Cis-Heptachlor epoxide	7.8	105	6.6	101
DDE-PP	15.0	89	12.7	79
DDT-OP	9.6	104	9.3	98
DDT-PP	6.1	103	7.0	99
Diazinon	8.6	99	8.7	90
Dichlorvos	9.1	102	6.0	97
p,p´-Dichlorobenzophenone	8.6	93	11.7	99
Dieldrin	9.7	101	10.0	96
Dimethoate	12.1	96	8.1	95
Endosulfan-Alpha	14.8	110	12.4	101
Endosulfan-Beta	24.4	107	19.3	98
Endrin	16.4	99	10.5	92
Fenitrothion	6.8	98	6.3	93
Hexachlorobutadiene	11.6	92	15.4	95
HCH-Alpha	6.2	104	6.4	98
HCH-Beta	14.1	104	11.4	102
HCH-Delta	9.8	108	11.3	100
HCH-Gamma	14.3	105	9.0	100
Heptachlor	27.7	114	26.0	110
Hexachlorobenzene	9.1	99	8.9	92
Irgarol 1051	19.1	95	15.3	88
Isodrin	9.2	104	11.4	98
Malathion	15.5	97	15.4	86
Pentachlorobenzene	12.0	100	13.7	93
Quinoxyfen	21.9	100	21.1	94
Simazine	13.6	105	8.2	97
TDE-PP	20.7	89	20.4	82
Terbutryn	13.6	95	12.0	87
Trans-Heptachlor epoxide	12.3	101	8.6	99
Trifluralin	15.8	101	10.0	98

As is clear in Table 6, the following conclusions can be drawn:

- The method used reaches all validation requirements and limits of quantitation for all pesticides as listed in Figure 2, except for the compound heptachlor and its epoxides.
- Excellent linearity (with $R^2 > 0.995$) was obtained over the concentration range tested (40–5000 pg/L).
- Recovery levels are between 79% and 114%.
- Residual standard deviation values were met for all compounds, except for heptachlor and its epoxides.
- Quantitation limits for the Water Framework Directive and CIP2 UK are met, except for heptachlor and its epoxides.

First results of validated method

After initial validation, daily quality controls are run by spiking river water at two levels, at the EQS level, and to control the sensitivity of the instrumentation a secondary spike at the LOQ level is monitored (Figure 3).

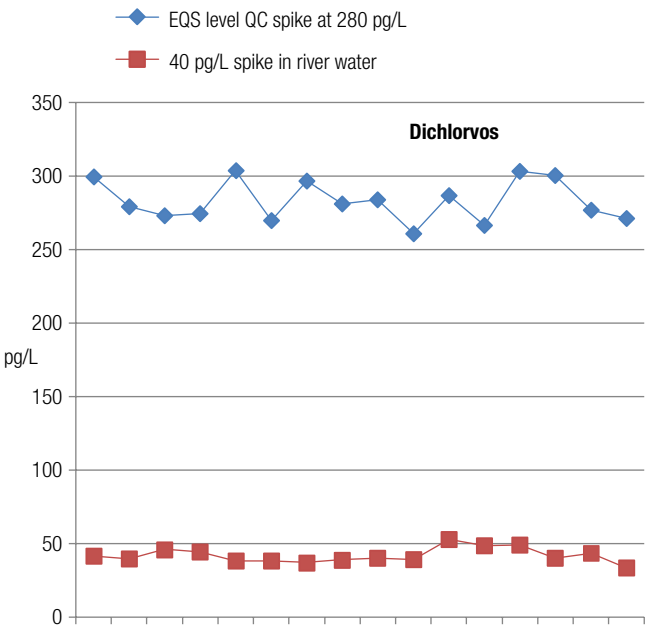

Figure 3. Results of daily repeated spikes of river water at the EQS and at the LOQ level for dichlorvos.

First data on UK surface waters

After the complete validation, samples have been analyzed according to the new detection limits established by the validation of the complete application (Figure 4).

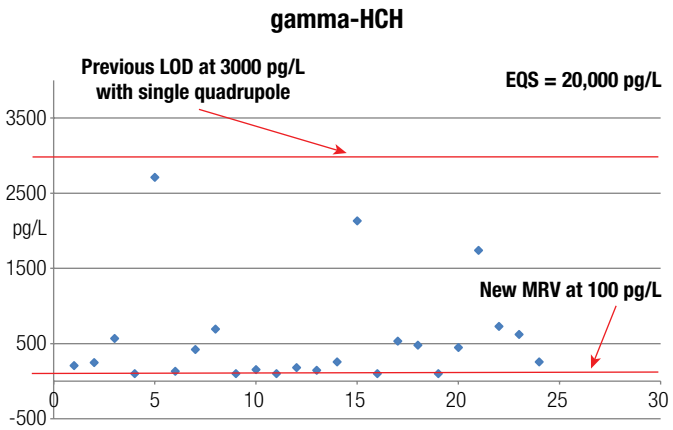

Figure 4. Blue diamonds represent the levels of gamma-HCH in surface waters across the UK.

Maintenance frequency of the validated method

- Liner and pre-column are replaced every 10 batches. A typical batch consists of 32 runs with calibration standards, AQCs, and samples.
- The septum is replaced after every two batches.
- Ion source cleaning is on average every three to four months.
- The analytical column is changed roughly once a year.

Conclusions

Sample preparation

This method is based on a sample amount of one liter. Liquid/liquid extraction is a classical, reliable, and easy extraction method, excellently suited for a large range of analytes water matrices.

GC and GC-MS/MS method and technology

Next to sample handling and preparation, a reliable and robust method has been developed for this set of analytes with the following crucial keys to success:

Large Volume PTV injection

Simply being able to inject a large volume of the extract limits the preconcentration of the sample to the amount of solvent volume, just enough to meet the target LOD. The methodology developed is robust and repeatable.

MS/MS

A secondary fragmentation and mass filtration allows for eliminating almost all background noise and yields much better signal-to-noise levels, resulting in lower detection limits.

RTA

The Retention Time Alignment tool provides an easy and fast method for adjusting the RT time shift generated by column trimming. The tool enables calculation and adjustment of the gas linear velocity to compensate for the difference in length between the two columns. It also allows balancing differences in the retention power between two columns.

Overall conclusions

- The method reaches all validation requirements and limits of quantitation for all pesticides, except for heptachlor and its epoxides.
- Excellent linearity was observed over the concentration range tested (40–5000 pg/L) with the coefficient of determination > 0.995.
- Recovery levels were between 80–114% for all compounds, meeting the EU Water Framework Directive regulations.
- Residual standard deviations were met for all compounds, except for heptachlor and its epoxides.
- Quantitation limits for the Water Framework Directive are met, except for heptachlor and its epoxides.

Acknowledgements

Thermo Fisher Scientific wishes to thank the experts in environmental analysis at the National Laboratory Services, Starcross Agency for sharing their data and validation results.

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