

# Meeting the requirements for the EU Water framework for the GC-MS amenable pesticides in water with LV-PTV-GC-MS/MS technology.

John Adey<sup>1</sup>, Greg Johnson<sup>2</sup>, Frans Schoutsen<sup>3</sup>, <sup>1</sup>National Laboratory Services, Starcross, Exeter, UK, <sup>2</sup>Thermo Fisher Scientific, Hemel Hempstead, UK, <sup>3</sup>ThermoFisher Scientific, Breda, The Netherlands

## ABSTRACT

**Purpose:** Presenting a validated method on pesticides in various water matrices with large volume injection into GC-MS after liquid-liquid extraction

**Methods:** Various pesticides were analyzed in the standards and matrix samples using a Thermo Scientific™ TSQ 8000 Evo triple quadrupole GC-MS/MS instrument coupled with a Thermo Scientific™ TRACE™ 1310 GC. Sample introduction was performed with a Thermo Scientific™ TriPlus™ RSH autosampler, using large volume PTV with a glass sintered liner and compound separation was achieved on a Thermo Scientific™ Trace GOLD TG-35MS 30 m x 0.25 mm I.D. x 0.25 µm film capillary column.

**Results:** The method is completely validated and samples are run daily in the lab.

## INTRODUCTION

The EU Water Framework directive was introduced in August 2013 amending EU directive 2000/60/EC and 2008/105/EC; laying down a strategy against the pollution of water to be applied to all European Union member states; it involves the identification of priority substances and the monitoring of different classes of contaminants; Includes the first watch list (used for future prioritization exercises); Member States have the flexibility to apply an EQS (environmental quality standard) for an alternative matrix or, where relevant, an alternative biota taxon, for example sub-phylum Crustacean, paraphylum "fish", class Cephalopoda or class Bivalvia (mussels and clams); The directive encourages the development of novel monitoring methods such as passive sampling and other tools.

CIP 2 UK regulations investigate the occurrence, sources and removal of trace substances in waste water treatment facility effluent. This regulation helps to establish priorities for remeditative action to ensure surface waters meet new Environmental Quality Standards (EQS). The CIP 1 program was managed by UK Water Industry Research (UKWIR) and implemented from 2010-2013. The CIP 2 program is a follow up program of sampling and analysis to be implemented between 2014 and 2020. The primary objective of CIP 2 is to identify and characterize sites where EQS levels are breached. In the program 70 priority substances were determined from 162 sewage treatment works (STW) effluents. 11 pharmaceuticals were also identified as priority monitoring candidates. It is important to note that EQS is defined for only 3 pharmaceutical compounds Diclofenac, E2 and EE2. All substances selected for monitoring analysis were detected previously in waste water effluent samples. The determined environmental concentrations of many priority substances in effluent exceeded EQS.

## CONSIDERATIONS

The limit of quantitation (LOQ) is the level at which can be accurately quantitated, while still achieving the required level of precision (typically 10 – 25%). As defined by the Directive, the LOQ is x2.15 the Limit of Detection (LOD). It is a very low level spike, making the RSD target very challenging, but in almost all cases we have achieved below 25% RSD for the LOQ (most compounds are comfortably below this).

Generally speaking, the Environmental Quality Standard (EQS) is defined as being 7 times the LOD. This value will be run routinely as part of our in-house quality control, with each batch of samples. It is also a very low level spike, but we comfortably achieve the RSD/Recovery targets for most compounds. The Directive values ref 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.

For all of the compounds the requirements are met except for heptachlor - and its epoxides. Currently there are no practical and affordable methodologies available to achieve these limits.

## MATERIALS AND METHODS

### Sample preparation

1 Litre of water sample is extracted with 25ml of dichloromethane and 5ml of iso-hexane. This ensures efficient extraction of a wide range of pesticides with different polarities.

The two solvents are taken off, combined and dried with sodium sulphate, and then concentrated down to 0.5ml using a Turbovap II system. Finally there is a solvent exchange step.

**Injection volume:** 60µl

### Instrument method

Oven Method	Use evaporation phase:	Yes	
Initial temperature:	40.0 °C	Use cleaning phase:	Yes
Initial hold time:	3.00 min	Use ramped pressure:	Yes
Number of ramps:	3	Transfer temperature delay:	0.10 min
Ramp 01 rate:	25.0 °C/min	Post-cycle temperature:	Cool Down
Ramp 01 final temperature:	180.0 °C	Injection pressure:	100.00 kPa
Ramp 01 hold time:	0.00 min	Injection time:	0.35 min
Ramp 02 rate:	5.0 °C/min	Injection flow:	40.0 mL/min
Ramp 02 final temperature:	260.0 °C	Injection backflush enable:	Off
Ramp 02 hold time:	5.00 min	Evaporation pressure:	100.00 kPa
Ramp 03 rate:	100.0 °C/min	Evaporation rate:	1.0 °C/sec
Ramp 03 final temperature:	300.0 °C	Evaporation temperature:	55 °C
Ramp 03 hold time:	5.00 min	Evaporation time:	1.00 min
<b>PTV mode:</b>	<b>Large Volume</b>	Evaporation flow:	50.0 mL/min
Temperature:	40 °C	Transfer pressure:	517.11 kPa
Split flow:	40.0 mL/min	Transfer rate:	14.5 °C/sec
Splitless time:	1.00 min	Transfer temperature:	320 °C
Purge flow:	40.0 mL/min	Transfer time:	3.50 min
Constant septum purge:	On	Cleaning rate:	14.5 °C/sec
Carrier mode:	Constant Pressure	Cleaning temperature:	370 °C
Carrier pressure:	100.00 kPa	Cleaning time:	10.00 min
Vacuum compensation:	On	Cleaning flow:	700.0 mL/min
Carrier gas saver flow:	10.0 mL/min	Cleaning backflush enable:	Off
Carrier gas saver time:	20.00 min		

Name	RT	ParentMass	ProductMass	CollisionEnergy	Name2	RT3	ParentMass3	ProductMass3	CollisionEnergy3
135-Trichlorobenzene	7.14	179.9	109.0	24	Chlorothalonil	17.53	265.8	170.0	24
135-Trichlorobenzene	7.14	179.9	109.0	14	Chlorothalonil	17.53	265.8	133.0	36
HCBD-C13	7.88	228.8	193.9	14	Aldrin	17.82	262.7	192.9	32
HCBD-C13	7.88	228.8	193.9	14	Aldrin	17.82	262.7	191.0	30
HCBD	7.88	222.9	187.9	14	Terbutryn	18.07	241.1	68.0	6
HCBD	7.88	224.9	189.9	14	Terbutryn	18.07	241.1	170.1	12
124-TCB	8.01	180.0	109.0	24	Chlorpyrifos-Ethyl	18.42	196.7	107.0	36
124-TCB	8.01	180.0	109.0	14	Chlorpyrifos-Ethyl	18.42	196.7	168.9	12
123-TCB-03	8.47	182.9	111.0	24	Fenitrothion	18.56	277.0	109.0	16
123-TCB-03	8.47	182.9	111.0	14	Fenitrothion	18.56	277.0	260.0	6
123-TCB	8.49	179.9	108.9	28	Parathion-DiO	18.78	301.1	83.0	24
123-TCB	8.49	179.9	108.9	18	Parathion-DiO	18.78	301.1	115.1	10
Dichlorvos-D6	8.98	115.0	83.0	6	Dicofol	19.31	192.9	123.0	28
Dichlorvos-D6	8.98	115.0	83.0	12	Dicofol	19.31	192.9	157.2	20
Dichlorvos	9.02	185.0	93.0	12	Dicofol	19.45	111.0	75.1	12
Dichlorvos	9.02	185.0	93.0	12	Dicofol	19.45	119.0	111.0	12
Pentachlorobenzene	11.55	249.8	214.8	16	Pendimethalin-D5	19.56	252.2	164.1	10
Pentachlorobenzene	11.55	249.8	214.8	16	Pendimethalin-D5	19.56	252.2	193.1	5
Trifluralin-D14	11.91	367.0	163.1	12	Pendimethalin	19.64	252.1	162.0	8
Trifluralin-D14	11.91	315.1	267.1	10	Pendimethalin	19.64	252.1	191.3	8
Trifluralin	12.02	366.1	266.0	10	cis-Heptachlor-Epoide	19.83	352.8	262.9	16
Trifluralin	12.02	366.1	264.1	8	cis-Heptachlor-Epoide	19.83	352.9	252.9	36
Hexachlorobenzene	14.06	283.8	213.8	30	Trans-Heptachlor-Epoide	20.12	352.9	253.0	16
Hexachlorobenzene	14.06	283.8	248.8	18	Trans-Heptachlor-Epoide	20.12	353.0	155.0	12
HCH-Alpha-D6	14.23	221.9	148.0	18	Chlorfenvinphos	20.28	266.9	159.0	16
HCH-Alpha-D6	14.23	221.9	185.0	18	Chlorfenvinphos	20.28	266.9	203.0	10
HCH-Alpha	14.37	182.8	146.7	12	Irgarol 1051	20.51	253.2	182.1	12
HCH-Alpha	14.37	218.8	183.0	8	Irgarol 1051	20.51	182.0	109.1	10
Diazinon	14.75	137.1	84.1	12	Endosulfan-alpha	21.24	240.6	205.9	14
Diazinon	14.75	179.1	121.5	28	Endosulfan-alpha	21.24	194.7	125.0	22
Atrazine-D5	14.94	205.1	127.1	14	DDE-PP	21.96	246.0	176.1	28
Atrazine-D5	14.94	205.1	105.0	10	DDE-PP	21.96	317.8	246.0	20
Atrazine	15.00	200.0	132.0	8	Dieldrin	22.33	262.8	227.8	16
Atrazine	15.00	215.0	58.1	12	Dieldrin	22.33	262.8	190.9	30
Simazine	15.18	172.7	138.0	6	Endrin	23.53	280.8	245.3	8
Simazine	15.18	186.0	91.0	8	Endrin	23.53	262.8	192.9	30
HCH-Gamma	15.58	180.9	145.0	14	DDE-PP	23.78	235.0	165.1	22
HCH-Gamma	15.58	180.9	109.0	26	DDE-PP	23.78	235.0	199.5	10
Dimethoate-D6	15.66	149.1	114.0	10	TDE-PP	24.20	235.0	165.1	20
Dimethoate-D6	15.66	149.1	131.1	14	TDE-PP	24.20	235.0	199.0	14
Dimethoate	15.76	143.0	110.3	10	Endosulfan-beta	24.61	240.6	205.8	12
Dimethoate	15.76	143.0	111.0	10	Endosulfan-beta	24.61	158.9	123.0	12
HCH-Beta	16.45	180.9	145.0	14	DDE-DB	25.16	243.0	172.2	24
HCH-Beta	16.45	218.7	183.0	8	DDE-DB	25.16	245.1	174.1	22
Heptachlor	16.75	271.8	236.9	12	Acidofen	25.19	264.0	194.1	14
Heptachlor	16.75	99.8	65.0	12	Acidofen	25.19	264.0	212.1	12
Alachlor-D13	16.78	172.2	137.2	26	DDE-PP	25.28	235.0	165.1	22
Alachlor-D13	16.78	200.2	172.2	10	DDE-PP	25.28	235.0	199.5	10
Alachlor	16.98	188.1	130.0	12	Quinoxifen	25.43	277.0	208.0	26
Alachlor	16.98	188.1	160.1	8	Quinoxifen	25.43	307.0	237.0	18
HCH-Delta	17.40	218.8	182.9	8	Bifenox	29.85	172.9	137.9	16
HCH-Delta	17.40	218.8	146.5	20	Bifenox	29.85	341.1	281.0	12

Figure 1-2 GC method and MS/MS Transitions

### Calibration spikes in Ultra High Purity water

The concentration values are as follows, in the 1 litre of UHP water and on column:

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

### Validation spikes in Surface River Water

The concentration values are as follows, in the 1 litre of surface river water and on column:

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

### Observations during method development and validation

Interferences at the very low levels, typically 80pg/l, from the sample matrix. For example, Dichlorvos, where the principal precursor ion is 109. Using this ion resulted in poor ion qualification at the sub 80pg/l level. Switching to the less abundant higher precursor masses 185 and 186.9, although less abundant resulted in better ion qualification.

Focusing of the Trichlorobenzenes at the front of the chromatogram. This proved difficult due to the 60ul enriched injection volume. We largely overcame this by using constant pressure mode, and a ramped pressure during the hot liner to cool column transfer stage.

Linearity above 5000pg/l (5ng/l) for some of the compounds was poor. This has restricted our calibration range, and therefore, has increased the requirement to dilute some of our samples, which does increase workload and cost.

## RESULTS

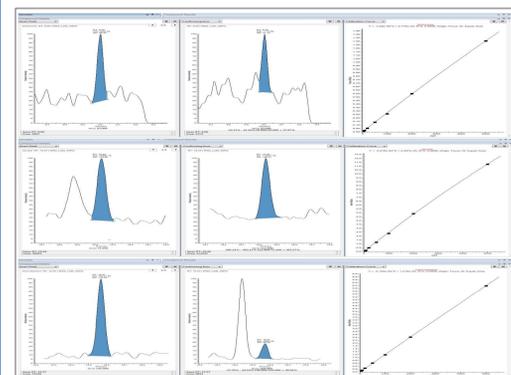


Figure 3 Quantifier and qualifier ion of dichlorvos, dicofol and g-HCH at 40pg/l level and calibration curves

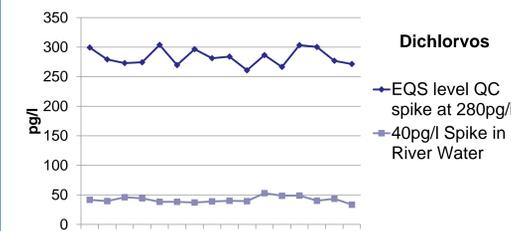


Figure 4 Graphical representation of the repeatability of dichlorvos at EQS spike and 40pg/L spike in river water

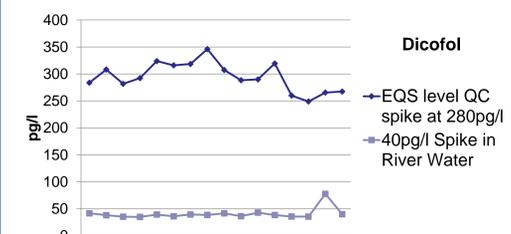


Figure 5 Graphical representation of the repeatability of dicofol at EQS spike and 40pg/L spike in river water

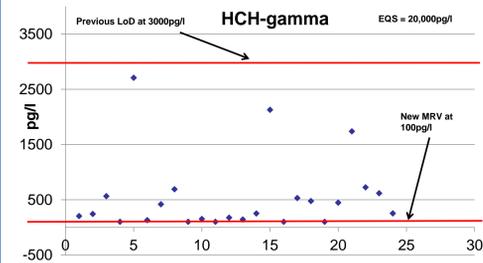


Figure 6 Graph representing measurements of river water samples for HCH-gamma.

The upper red line represents the previous LOD when analyzing with GC single quad, the lower red line represents the new quantitation limit. The green dots are actual measurements of various samples.

## VALIDATION:

### Statistical evaluation over 11 batches

As part of the validation, 11 batches of validation were analyzed. All the spiked samples were extracted and run in duplicate. Over the 11 batches the residual standard deviation target is 25%, and the recovery target is 100% ± 20%. Due to background issues, Chlorothalonil and Pendimethalin were spiked at higher levels for the LOD i.e. the EQS 280pg/l spike was used for LOD purposes. This level is still within the requirement of the directive

	% RSD at 80pg/l in River Water	% Recovery at 80pg/l in River Water	%RSD at EQS (280pg/l) in River Water	% Recovery at EQS (280pg/l) in River Water
1,2,3-Trichlorobenzene	26.9	91	10	93
1,2,4-Trichlorobenzene	23	103	6.4	94
1,3,5-Trichlorobenzene	12	104	17.5	101
Acidofen	16.8	99	18.8	96.5
Alachlor	6.5	99	6.2	94
Aldrin	8.4	98	7.6	94
Atrazine	15	96	6.8	94
Bifenox	16.5	101	16.6	95
Chlorfenvinphos	17	97	23.3	94
Chlorpyrifos	24.9	108	13.3	95
Chlorothalonil	No Data	No Data	No Data	No Data
Cis-Heptachlor epoxide	7.8	105	6.6	101
DDE-PP	15	89	12.7	79
DDT-OP	9.6	104	9.3	98
DDT-PP	6.1	103	7	99
Diazinon	8.6	99	8.7	90
Dichlorvos	9.1	102	6	97
Dicofol	8.6	93	11.7	99
Dieldrin	9.7	101	10	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	9.1	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	100
Heptachlor	27.7	114	26	110
Heptachlorobenzene	9.1	99	8.3	93
Irgarol 1051	19.1	95	15.3	88
Isodrin	9.2	104	11.4	98
Malathion	15.5	97	15.4	86
Pendimethalin	No data	No Data	No Data	No Data
Pentachlorobenzene	12	100	13.7	93
Quinoxifen	21.9	100	21.1	94
Simazine	13.6			