

# Automated sample preparation followed by sensitive analysis by GC-MS/MS for environmental contaminants in surface waters

## Authors

Cees Bijsterbosch,<sup>1</sup> Cedric Wissel,<sup>2</sup> Cristian Cojocariu,<sup>3</sup> and Inge de Dobbeleer<sup>4</sup>

<sup>1</sup>Het Waterlaboratorium, Haarlem, The Netherlands; <sup>2</sup>Interscience BV, Breda, the Netherlands; <sup>3</sup>Thermo Fisher Scientific, Runcorn, UK;

<sup>4</sup>Thermo Fisher Scientific, Breda, The Netherlands

## Keywords

Liquid-liquid extraction, automation, surface waters, environmental contaminants, advanced electron ionization, AEI, GC-MS/MS

## Goal

To establish the feasibility of automating the workflow for analyzing semi-volatiles with GC-MS/MS in surface waters.

## Introduction

Environmental contaminants remain a constant cause for concern with the public and there is a need for many laboratories to analyze samples in a fast and cost-effective way. Laboratories look for options to save on solvent costs and to minimize sample preparation time. However, time-saving and cost-driven measurements should not compromise the analytical results in terms of sensitivity, robustness, or quality controls. Consequently, to be able to minimize costs and efforts from the laboratory, the ideal analytical system typically needs to demonstrate: the capability of handling the automation of sample preparation, the flexibility of injection modes (such as large volume injections), and the sensitivity required for detection at the ultra low level. In this study we demonstrate the Thermo Scientific™ TSQ™ 9000 triple quadrupole mass spectrometer configured with the Advanced Electron Ionization (AEI) source and Thermo Scientific™ Triplus™ RSH™ autosampler for the automated sample preparation and subsequent detection of contaminants at ultra low levels in a surface water matrix.<sup>1,2</sup>

## Experimental

### Sample preparation

Automated sample preparation was performed with the Triplus RSH autosampler equipped with several syringe types capable of introducing solvents and internal standards to the sample. The autosampler was also

equipped with a vortex mixer to facilitate more efficient extraction. A separate 200  $\mu\text{L}$  syringe with a side hole needle was utilized for injecting the extracted sample. Sample preparation was completely automated. A short overview of the procedure is given below:

1. Sample (10 mL) was pipetted into a 20 mL headspace vial
2. A mix of internal standards was added (see below)
3. Pentane (2 mL) was added as the extraction solvent
4. The sample was vortexed for 1 minute at a speed of 2000 cycles/min
5. Five minutes of phase separation waiting time was followed by a Large Volume PTV injection of 50  $\mu\text{L}$

This procedure reduced the handling time of the lab technician significantly, and moreover, the amount of solvent needed for extraction is very low. The analyst only needed to pipette the sample into the headspace vial. The robotic sample handling procedure was developed and implemented by SampleQ™ (Breda, The Netherlands) in collaboration with Het Waterlaboratorium.<sup>6,7</sup>

The following compounds were used as internal standards:

- 2,4 dichlorotoluene
- D10-acenaphthene
- D10-anthracene
- D10-phenanthrene
- D12-benzo (a) pyrene
- D12-chrysene
- D3-PCB101
- D4-DDD
- D8-naphthalene

### GC-MS experimental conditions

Gas chromatography-mass spectrometry (GC-MS) was performed on a Thermo Scientific™ TRACE 1310™ gas chromatograph equipped with a Thermo Scientific™ Instant Connect Programmable Temperature Vaporizing (PTV) injector module and linked to the TSQ 9000 triple quadrupole mass spectrometer with AEI source. Separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-5-SilMS, 60 m  $\times$  0.25  $\mu\text{m}$   $\times$  0.25 mm ID capillary column (P/N 26096-1540). A Thermo Scientific™ LinerGOLD™ GC Liner (P/N 45352060) with sintered lining was used for the large volume injection.<sup>3-5</sup>

Experimental parameters are listed in Tables 1 and 2. The compounds, retention times, and SRM transitions are listed in Appendix A.

**Table 1. GC oven and injection methods.**

<b>Oven Method</b>	
Initial temperature:	60 °C
Initial hold time:	5.00 min
Number of ramps:	1
Ramp rate:	10.0 °C/min
Ramp final temperature:	300 °C
Ramp hold time:	15.00 min
<b>PTV</b>	
Injection speed:	5 $\mu\text{L/s}$
Injection volume:	50 $\mu\text{L}$
PTV mode:	Large volume
Temperature:	40 °C
Split flow:	40.0 mL/min
Splitless time:	2.00 min
Purge flow:	5.0 mL/min
Carrier mode:	Constant flow
Carrier flow:	1.80 mL/min
Vacuum compensation:	On
Transfer temperature delay:	2.00 min
Post-cycle temperature:	Maintain
Injection time:	0.10 min
Injection flow:	20 mL/min
Transfer rate:	5.0 °C/s
Transfer temperature:	320 °C
Transfer time:	3.00 min
Cleaning rate:	14.5 °C/s
Cleaning temperature:	340 °C
Cleaning time:	10.00 min
Cleaning flow:	75.0 mL/min

**Table 2. MS method parameters.**

MS acquisition type:	timed-SRM
Instrument type:	TSQ 9000 GC-MS/MS system
MS transfer line:	300 °C
Ion source temperature:	280 °C
Ionization mode:	EI with AEI source
Quadrupole resolution:	0.7 Da FWHM (both Q1 and Q3)

## Data processing

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software, which is a single software platform that allows for fast data acquisition, customized templates, and automatic report generation. Compound databases for SRM are included, and the software's *Method Forge* ensures easy access to hundreds of molecules in seconds.

## Results and discussion

In the experiments below a series of spiked water samples were used to determine the linearity of the compounds of interest:

- Level 1: 5 ng/L water or 1.25 pg absolute injected
- Level 2: 20 ng/L water or 5 pg absolute injected
- Level 3: 100 ng/L water or 25 pg absolute injected
- Level 4: 200 ng/L water or 50 pg absolute injected
- Level 5: 400 ng/L water or 100 pg absolute injected
- Level 6: 600 ng/L water or 150 pg absolute injected
- Level 7: 800 ng/L water or 200 pg absolute injected
- Level 8: 1000 ng/L water or 250 pg absolute injected

The autosampler also contained a series of 10 vials with surface water spiked at a level of 100 ng/L water, plus surface water blanks. A series of 10 vials spiked at a lower level of 10 ng/L was also added to the sequence.

In addition, a quality control (QC) sample was acquired before and after the surface water samples at a level of 100 ng/L water. All compounds were added to either a water blank or the surface water directly and all extraction was performed by the automated procedure.

For evaluating the data the following rules were applied:

- All 60 compounds are certified and traceable according to the following rules and regulations: Guide 34:2000, ISO17025:2005, ISO 9001:2008
- All results are corrected for the blank deionized water and blank surface waters
- All compounds identified conform to NTA 8379. This is a Dutch pre-standard of the ISO standard "Water quality—Multi-class methods—Part 1: Guidance for the identification of target compounds by gas and liquid chromatography and mass spectrometry"

This sequence was repeated several times. In total, 50 injections were performed under these conditions to establish linearity, repeatability, and instrument detection limits.

## Calibration curves

For all compounds, linear calibration responses were achieved, with correlation coefficients of a minimum of  $R^2 = 0.995$  and residual values below 25% for all compounds (Figures 1–3, Table 3).

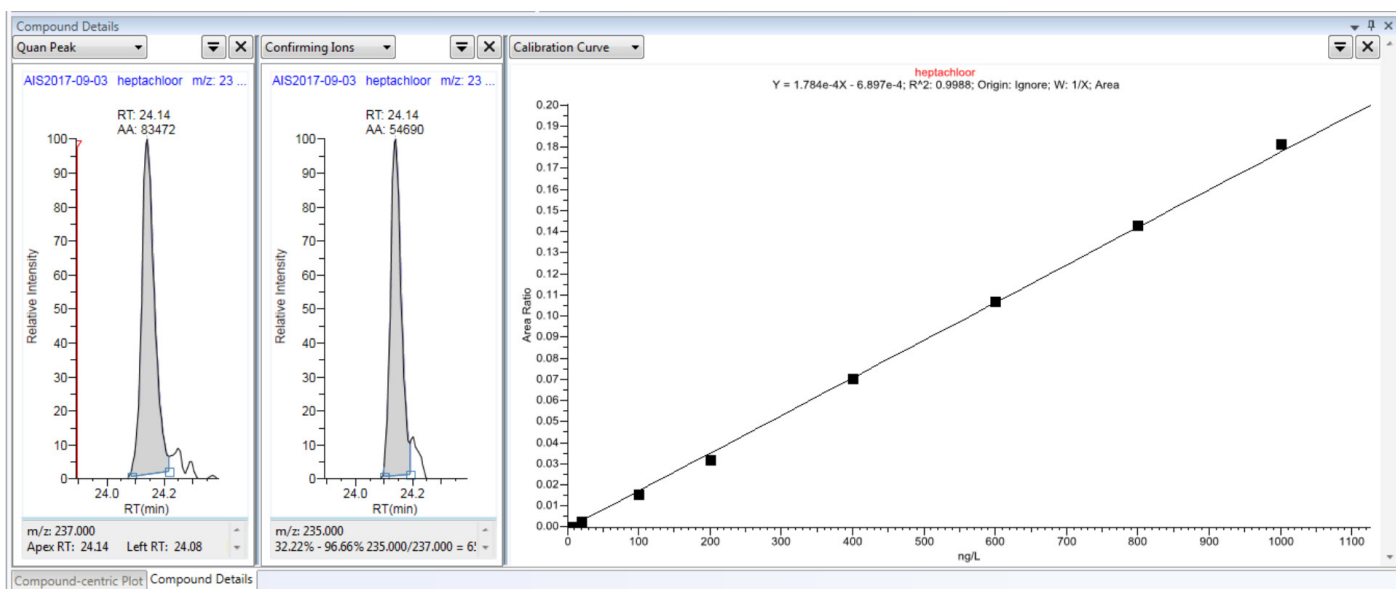


Figure 1. Chromatograms of both quantification (left) and confirmation (right) SRM transitions of heptachlor at the lowest level of 5 ng/L or 1.25 pg absolute amount on column. The calibration curve of heptachlor over 5 to 1000 ng/L is also shown.

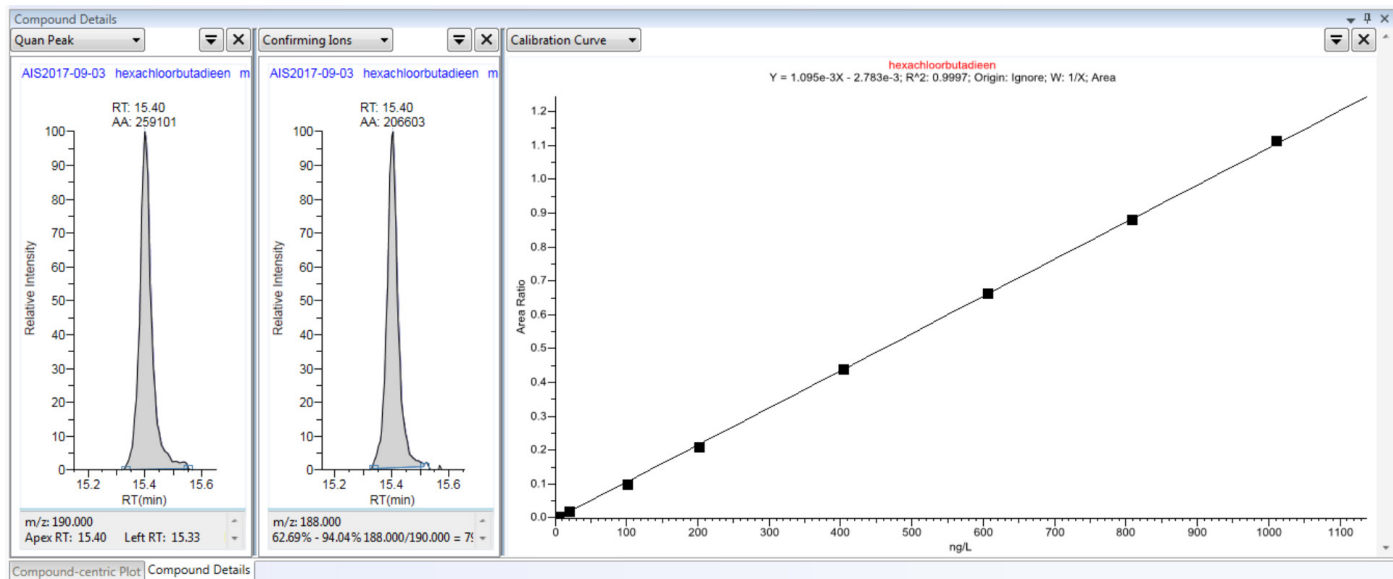


Figure 2. Chromatograms of both quantification (left) and confirmation (right) SRM transitions of hexachlorobutadiene at the lowest level of 5 ng/L or 1.25 pg absolute on column. The calibration curve of hexachlorobutadiene over 5 to 1000 ng/L is also shown.

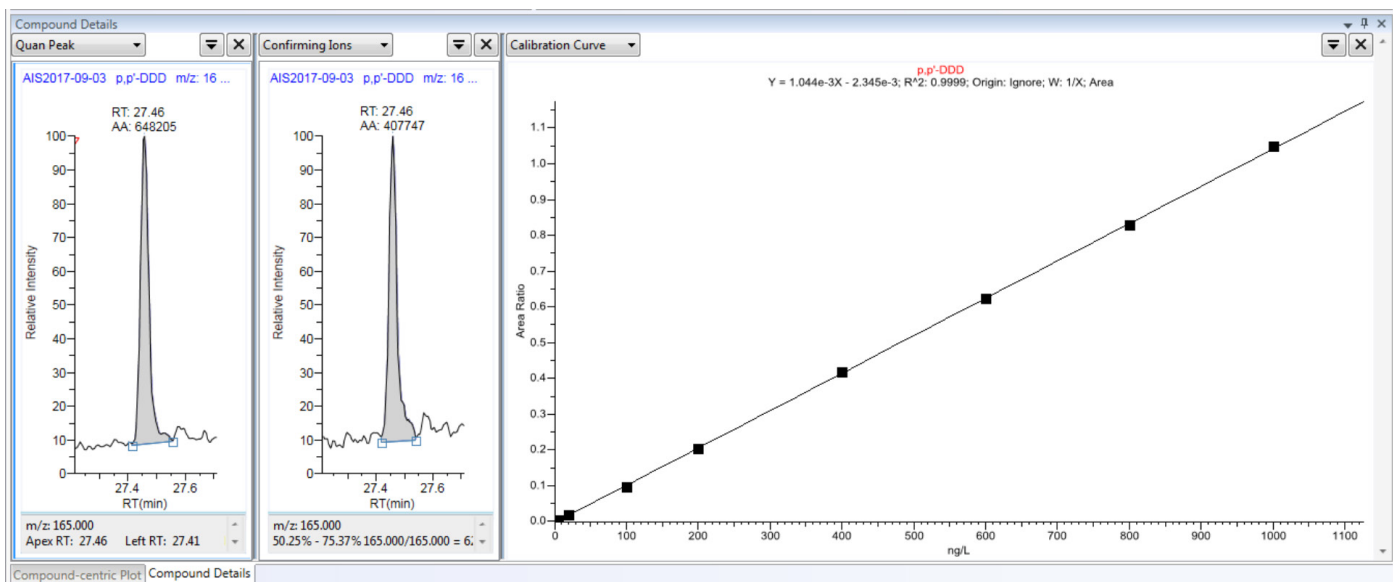


Figure 3. Chromatograms of both quantification (left) and confirmation (right) SRM transitions of p,p'-DDD at the lowest level of 5 ng/L or 1.25 pg absolute on column. The calibration curve of p,p'-DDD over 5 to 1000 ng/L is also shown.

Table 3. Examples of compound residuals at various levels of the calibration curve.

Compound Residual	Level 5 ng/L	Level 20 ng/L	Level 100 ng/L	Level 200 ng/L	Level 400 ng/L	Level 600 ng/L	Level 800 ng/L	Level 1000 ng/L
heptachlor	+23.76%	-7.50%	-10.21%	-9.01%	-0.49%	+0.61%	+0.75%	+2.08%
hexachlorobutadiene	+10.72%	-2.02%	-6.96%	-3.27%	+0.10%	+0.29%	+0.03%	+1.10%
p,p'-DDD	+6.58%	-2.21%	-4.31%	-1.13%	+0.83%	+0.01%	-0.51%	+0.74%

## Repeatability

The peak area repeatability was determined by spiking the same surface water at a level of 100 ng/L and performing n = 10 subsequent injections. The samples

were placed inside the autosampler for automated extraction and were subsequently analyzed using the TSQ 9000 GC-MS/MS system (Figures 4 and 5, Table 4).



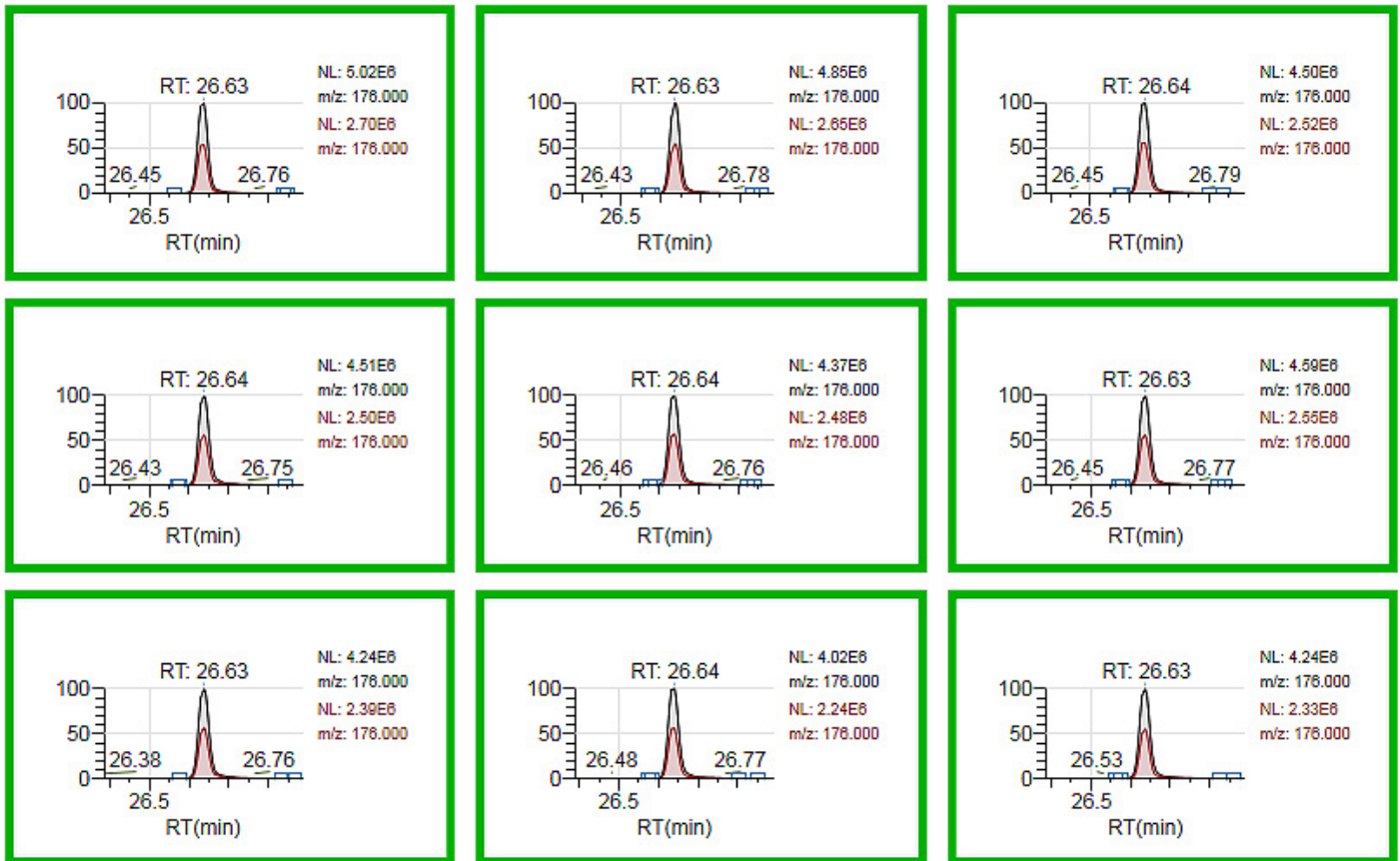


Figure 4. p,p'-DDE in surface water samples, showing 9 of the 10 repeat extractions and injections.

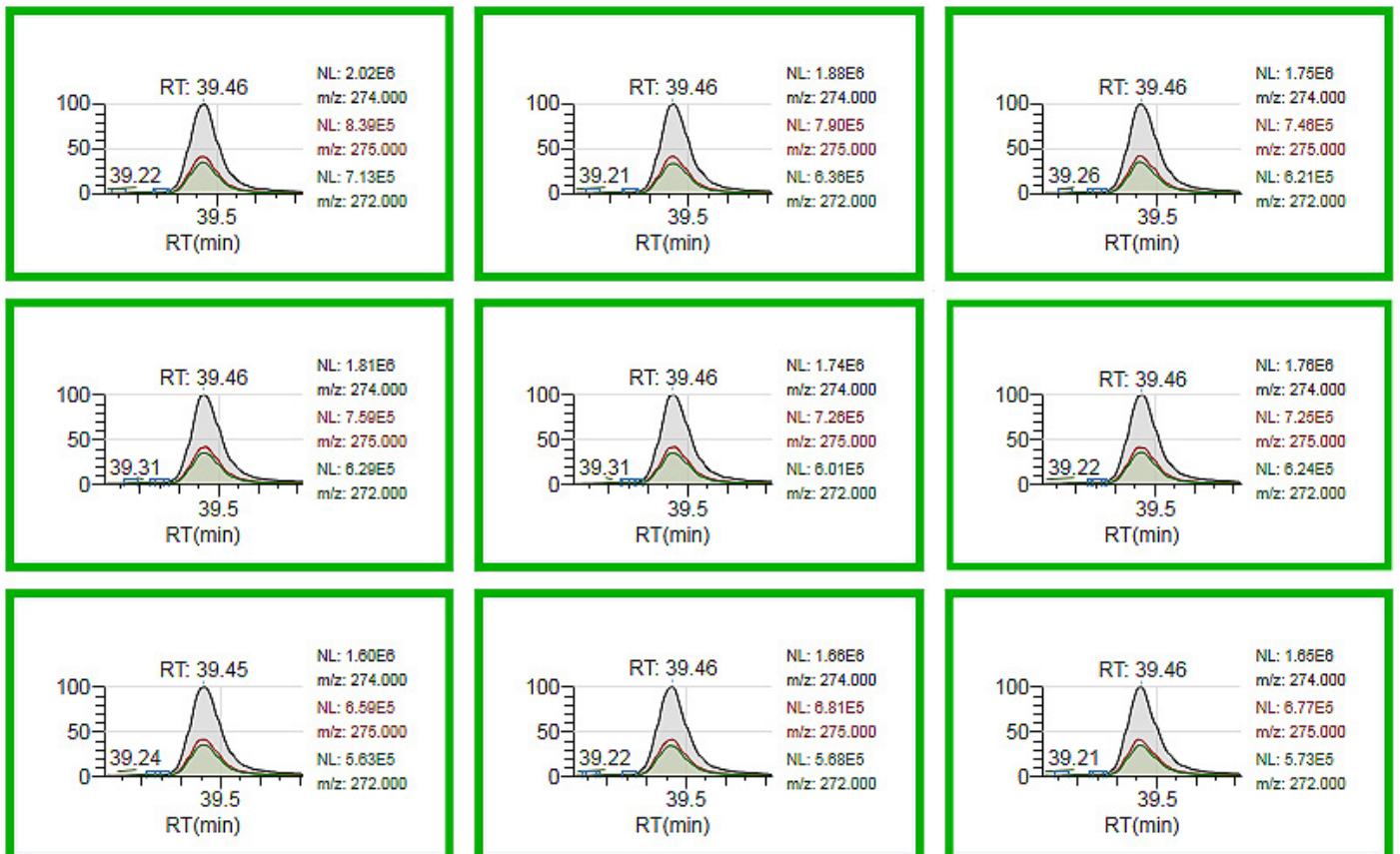


Figure 5. Benzo(ghi)perylene in surface water samples, showing 9 of the 10 repeat extractions and injections.

**Table 4. Compound list with % RSD on ten extractions plus injections of spiked surface water at 100 ng/L, the regression coefficients of the calibration curve, and the IDL based on repeated injections of a 10 ng/L spike to surface water.**

Compound	RT	% RSD at 100 ng/L	R <sup>2</sup>	IDL in ng/L
1,3-dichlorobenzene	12.14	0.81	0.9997	0.68
1,4-dichlorobenzene	12.27	1.13	0.9998	0.63
1,2-dichlorobenzene	12.59	1.00	0.9998	0.40
hexachloroethane	13.28	3.39	0.9995	1.03
1,3,5-trichlorobenzene	14.15	1.07	0.9997	0.84
1,2,4-trichlorobenzene	14.88	1.51	0.9997	1.51
naphthalene	15.10	0.87	0.9998	4.55
hexachlorobutadiene	15.40	1.16	0.9997	0.22
1,2,3-trichlorobenzene	15.46	1.58	0.9996	1.33
1,2,3,4-tetrachlorobenzene	17.23	0.73	0.9997	0.92
1,2,4,5-tetrachlorobenzene	17.94	0.79	0.9997	1.28
acenaphthylene	19.05	1.91	0.9991	3.25
acenaphthene	19.45	0.66	0.9995	1.22
pentachlorobenzene	19.74	1.30	0.9997	1.20
fluorene	20.73	1.41	0.9992	8.63
diphenylamine	21.09	1.45	0.9971	1.93
alpha-HCH	21.91	2.26	0.9994	1.02
hexachlorobenzene	21.98	3.76	0.9994	0.80
beta-HCH	22.50	3.23	0.9975	1.36
gamma-HCH	22.62	3.83	0.9987	0.91
propylamide	22.79	2.36	0.9992	3.68
pyrimethanil	23.00	1.52	0.9999	0.97
phenanthrene	23.02	1.36	0.9995	2.70
anthracene	23.14	1.94	0.9993	2.53
PCB-28	23.80	0.79	0.9995	0.51
alachlor	23.93	2.49	0.9989	2.12
heptachlor	24.13	1.98	0.9988	1.05
PCB-52	24.46	1.09	0.9997	0.83
aldrin	24.87	3.75	0.9997	0.74
cyprodinil	25.39	1.40	0.9995	1.78
cis-heptachlor epoxide	25.61	2.02	0.9991	2.67
trans-heptachlor epoxide	25.71	5.48	0.9861	17.84
fluoranthene	25.86	1.50	0.9995	5.16
PCB-101	26.11	1.73	0.9997	0.79
alpha-endosulfan	26.36	3.62	0.9994	3.01
pyrene	26.40	3.72	0.9993	4.14
p,p'-DDE	26.63	1.28	0.9998	0.81
kresoxim-methyl	26.70	2.38	0.9992	1.61
bupirimate	26.72	3.13	0.9978	1.27
dieldrin	26.87	3.67	0.9990	3.49
endrin	27.30	3.11	0.9991	5.64
PCB-118	27.30	1.76	0.9995	0.53

**Table 4. Compound list with % RSD on ten extractions plus injections of spiked surface water at 100 ng/L, the regression coefficients of the calibration curve, and the IDL based on repeated injections of a 10 ng/L spike to surface water (continued).**

Compound	RT	% RSD at 100 ng/L	R <sup>2</sup>	IDL in ng/L
p,p'-DDD	27.45	2.09	0.9999	1.37
beta-endosulfan	27.50	2.29	0.9992	4.04
PCB-138	27.67	1.69	0.9996	0.36
p,p'-DDT	28.15	5.56	0.9974	8.79
PCB-153	28.20	1.18	0.9996	2.30
piperonyl-butoxide	28.42	2.35	0.9985	3.71
fluxapyroxad	28.94	1.83	0.9989	1.78
benzo(a)anthracene	29.27	4.58	0.9992	3.51
chrysene	29.36	4.23	0.9996	3.17
PCB-180	29.40	3.65	0.9994	0.89
isopyrazam	30.48	5.90	0.9988	1.32
benzo(b)fluoranthene	32.23	1.41	0.9992	4.22
benzo(bk)fluoranthene	32.30	2.19	0.9990	2.98
benzo(k)fluoranthene	32.32	2.38	0.9995	1.25
benzo(a)pyrene	33.32	1.56	0.9997	1.63
indeno(123-cd)pyrene	38.08	2.15	0.9984	1.32
dibenzo(ah)anthracene	38.21	1.49	0.9978	2.61
benzo(ghi)perylene	39.46	2.49	0.9987	1.38

## Conclusions

The experimental results show that automated sample preparation with the TriPlus RSH autosampler combined with the TSQ 9000 triple quadrupole GC-MS/MS system with the AEI source is a powerful, cost-saving configuration:

- Can be completely automated with excellent repeatabilities
- Saves solvents and avoids exposure to solvents for the analyst
- Provides a very high level of sensitivity and excellent linearity
- Allows the laboratory to save time on intensive sample preparation

The easy-to-use method has low detection limits, excellent repeatability, and linearity for a large number of contaminants in surface water samples.

## Acknowledgments

Thermo Fisher Scientific wishes to thank the experts at Het Waterlaboratorium in Haarlem, the Netherlands, and the team from SampleQ. Special thanks to Cees Bijsterbosch for designing the experiments and sharing the data, and to Cedric Wissel for the automatization of the sample preparation.

## References

1. Analysis of emerging persistent organic pollutants using GC-MS/MS; Kalachova *et al.* SETAC, Berlin 2012.
2. Ziegenhals, K.; Hubschmann, H.J. Fast-GC/HRMS to quantify the EU priority PAH. *J. Sep. Sci.* **2008**, *31*, 1779 – 1786.
3. Thermo Scientific Application Brief AB52998 - Introducing AutoSRM: MRM Simplicity for High Performance Results; Cole J.
4. REGULATION (EC) No 2002/657 on analytical performance criteria.
5. Pesticides Method Reference, 2nd ed. 2011, Thermo Fisher Scientific, Austin, TX, USA, P/N 120390.
6. [www.sampleq.nl](http://www.sampleq.nl)
7. [www.hetwaterlaboratorium.nl](http://www.hetwaterlaboratorium.nl)

## Appendix A. Compound list, retention times, and SRM transitions.

Number	Name	RT (min)	Precursor Mass (Da)	Product Mass (Da)	Collision Energy (V)
1	1,3-dichlorobenzene	12.17	146	111	15
2	1,3-dichlorobenzene	12.17	148	113	15
3	1,4-dichlorobenzene	12.30	146	111	15
4	1,4-dichlorobenzene	12.30	148	113	15
5	1,2-dichlorobenzene	12.63	146	111	15
6	1,2-dichlorobenzene	12.63	148	113	15
7	hexachloroethane	13.32	201	166	14
8	hexachloroethane	13.32	199	164	10
9	2,4-dichlorotoluene (IS)	13.96	125	89	10
10	2,4-dichlorotoluene (IS)	13.96	127	90	25
11	1,3,5-trichlorobenzene	14.17	180	145	20
12	1,3,5-trichlorobenzene	14.17	180	109	20
13	1,2,4-trichlorobenzene	14.90	180	109	20
14	1,2,4-trichlorobenzene	14.90	180	145	20
15	d8-naphthalene (IS)	15.06	136	108	35
16	d8-naphthalene (IS)	15.06	136	82	35
17	naphthalene	15.11	128	102	20
18	naphthalene	15.11	128	127	25
19	hexachlorobutadiene	15.40	225	190	16
20	hexachlorobutadiene	15.40	223	188	16
21	1,2,3-trichlorobenzene	15.47	180	109	20
22	1,2,3-trichlorobenzene	15.47	180	145	20
23	1,2,3,4-tetrachlorobenzene	17.22	214	108	30
24	1,2,3,4-tetrachlorobenzene	17.22	216	181	20
25	1,2,4,5-tetrachlorobenzene	17.93	214	108	30
26	1,2,4,5-tetrachlorobenzene	17.93	216	181	20
27	acenaphthylene	19.04	152	126	25
28	acenaphthylene	19.04	152	102	30
29	d10-acenaphthene	19.36	164	162	12
30	d10-acenaphthene	19.36	162	160	18
31	acenaphthene	19.44	153	152	25
32	acenaphthene	19.44	153	151	25
33	pentachlorobenzene	19.74	250	215	25
34	pentachlorobenzene	19.74	248	213	25
35	fluorene	20.71	165	163	30
36	fluorene	20.71	166	164	30
37	diphenylamine	21.06	169	168	20
38	diphenylamine	21.06	169	167	20
39	alpha-HCH	21.91	181	145	15
40	alpha-HCH	21.91	217	181	8
41	hexachlorobenzene	21.98	284	249	20
42	hexachlorobenzene	21.98	284	214	20



## Appendix A. Compound list, retention times, and SRM transitions (continued).

Number	Name	RT (min)	Precursor Mass (Da)	Product Mass (Da)	Collision Energy (V)
43	beta-HCH	22.48	217	181	8
44	beta-HCH	22.48	219	183	8
45	gamma-HCH	22.63	181	145	15
46	gamma-HCH	22.63	217	181	8
47	propyzamide	22.76	175	147	15
48	propyzamide	22.76	173	109	18
49	d10-phenanthrene	22.96	188	160	20
50	d10-phenanthrene	22.96	188	158	34
51	d10-phenanthrene	22.96	184	156	22
52	pyrimethanil	22.97	199	198	10
53	pyrimethanil	22.97	198	118	35
54	phenanthrene	23.02	178	152	20
55	phenanthrene	23.02	178	176	20
56	d10-anthracene	23.10	188	160	18
57	d10-anthracene	23.10	188	158	32
58	d10-anthracene	23.10	184	156	20
59	anthracene	23.14	178	176	20
60	anthracene	23.14	178	152	20
61	PCB-28	23.81	256	186	20
62	PCB-28	23.81	258	186	20
63	alachlor	23.92	188	160	10
64	alachlor	23.92	188	131	18
65	heptachlor	24.14	272	237	15
66	heptachlor	24.14	270	235	15
67	PCB-52	24.46	290	220	20
68	PCB-52	24.46	292	220	20
69	aldrin	24.88	261	191	30
70	aldrin	24.88	263	193	30
71	aldrin	24.88	265	193	32
72	cyprodinil	25.38	224	208	30
73	cyprodinil	25.38	225	210	25
74	cis-heptachloroepoxide	25.61	351	261	15
75	cis-heptachloroepoxide	25.61	353	263	15
76	cis-heptachloroepoxide	25.61	353	317	10
77	trans-heptachloroepoxide	25.70	263	228	15
78	trans-heptachloroepoxide	25.70	353	253	20
79	trans-heptachloroepoxide	25.70	353	289	10
80	trans-heptachloroepoxide	25.70	263	193	30
81	fluoranthene	25.86	202	200	30
82	fluoranthene	25.86	202	152	30
83	d3-PCB101	26.10	294	259	10
84	d3-PCB101	26.10	259	187	35

## Appendix A. Compound list, retention times, and SRM transitions (continued).

Number	Name	RT (min)	Precursor Mass (Da)	Product Mass (Da)	Collision Energy (V)
85	PCB-101	26.12	326	256	20
86	PCB-101	26.12	324	254	20
87	alpha-endosulfan	26.37	241	206	10
88	alpha-endosulfan	26.37	243	208	10
89	pyrene	26.40	202	200	30
90	pyrene	26.40	202	176	30
91	pyrene	26.40	202	152	30
92	p,p'-DDE	26.64	246	176	25
93	p,p'-DDE	26.64	248	176	20
94	kresoxim-methyl	26.69	206	131	15
95	kresoxim-methyl	26.69	206	116	15
96	bupirimate	26.71	273	193	10
97	bupirimate	26.71	316	208	10
98	dieldrin	26.89	263	193	30
99	dieldrin	26.89	263	191	32
100	dieldrin	26.89	265	193	28
101	dieldrin	26.89	277	206	20
102	endrin	27.31	263	193	32
103	endrin	27.31	263	228	20
104	endrin	27.31	263	191	28
105	PCB-118	27.31	324	254	20
106	PCB-118	27.31	326	256	20
107	d4-DDD	27.41	243	173	20
108	d4-DDD	27.41	245	173	25
109	p,p'-DDD	27.46	235	165	20
110	p,p'-DDD	27.46	237	165	20
111	beta-endosulfan	27.51	241	206	10
112	beta-endosulfan	27.51	243	208	10
113	PCB-138	27.69	360	290	25
114	PCB-138	27.69	358	288	25
115	p,p'-DDT	28.17	235	165	20
116	p,p'-DDT	28.17	237	165	20
117	PCB-153	28.21	360	290	25
118	PCB-153	28.21	358	288	25
119	piperonyl-butoxide	28.42	176	131	15
120	piperonyl-butoxide	28.42	176	103	10
121	fluxapyroxad	28.93	381	159	6
122	fluxapyroxad	28.93	159	139	8
123	benzo(a)anthracene	29.28	228	226	30
124	benzo(a)anthracene	29.28	228	202	5
125	d12-chrysene	29.31	240	236	32
126	d12-chrysene	29.31	240	238	14

Appendix A. Compound list, retention times, and SRM transitions (continued).

Number	Name	RT (min)	Precursor Mass (Da)	Product Mass (Da)	Collision Energy (V)
127	chrysene	29.37	228	226	30
128	chrysene	29.37	228	202	5
129	PCB-180	29.42	394	324	25
130	PCB-180	29.42	392	322	25
131	isopyrazam	30.49	159	139	8
132	isopyrazam	30.49	303	262	16
133	benzo(b)fluoranthene	32.25	252	250	30
134	benzo(b)fluoranthene	32.25	252	226	30
135	benzo(bk)fluoranthene	32.33	252	250	30
136	benzo(bk)fluoranthene	32.33	252	226	30
137	benzo(k)fluoranthene	32.34	252	250	25
138	benzo(k)fluoranthene	32.34	252	226	32
139	d12-benzo(a)pyrene	33.26	264	260	38
140	d12-benzo(a)pyrene	33.26	260	256	38
141	d12-benzo(a)pyrene	33.26	264	236	30
142	benzo(a)pyrene	33.35	252	250	25
143	benzo(a)pyrene	33.35	252	226	30
144	indeno(123-cd)pyrene	38.13	276	274	35
145	indeno(123-cd)pyrene	38.13	276	275	25
146	indeno(123-cd)pyrene	38.13	276	248	40
147	dibenzo(ah)anthracene	38.25	278	276	35
148	dibenzo(ah)anthracene	38.25	278	252	25
149	dibenzo(ah)anthracene	38.25	139	125	25
150	d12-benzo(ghi)perylene	39.37	288	284	50
151	d12-benzo(ghi)perylene	39.37	288	286	35
152	benzo(ghi)perylene	39.52	276	274	45
153	benzo(ghi)perylene	39.52	276	275	25
154	benzo(ghi)perylene	39.52	276	272	60

Find out more at [thermofisher.com/TSQ9000](https://thermofisher.com/TSQ9000)

©2018 Thermo Fisher Scientific Inc. All rights reserved. SampleQ is a trademark of SampleQ. All other trademarks are the property of Thermo Fisher Scientific. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. **AN10591-EN 0318M**

**ThermoFisher**  
SCIENTIFIC