Application Note

Environmental

Analysis of Semivolatile Compounds in Water

Low-density liquid-liquid microextraction (LDME) with the Agilent 7010 Triple Quadrupole GC/MS

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Abstract

A simple, fast, inexpensive, robust, and sensitive method was developed to facilitate the analysis of multiple semivolatile compounds (sVOCs) in different types of water samples. The method is based on low-density liquid-liquid microextraction (LDME) coupled with detection using the sensitive Agilent 7010 triple quadrupole GC/MS (GC/TQ).

LDME GC/TQ method linearity, repeatability (n = 10 spiked blanks analyzed in one day), reproducibility (n = 150 spiked real-world samples, analyzed over one year by different analysts), recovery, sensitivity, and carryover were determined following ISO 17025 validation guidelines. Sub-ng/L limits of detection (LOD) and limits of quantitation (LOQ), meeting EU directives for sVOCs, were routinely and confidently achieved. Analyses at sub-ng/L levels are challenging due to the potential for cross-laboratory cross-contamination. The LDME GC/TQ method minimizes the chance of cross-contamination because the LDME supplies are inexpensive and disposable, and the Agilent GC inlets used ensure low carryover.

Interlaboratory proficiency tests carried out on drinking (tap) and surface water samples demonstrated that results obtained from LDME are comparable to those obtained using Stir Bar Sorptive Extraction (SBSE) and Solid Phase Extraction (SPE). The proficiency testing also studied matrix effects on method results in surface water samples containing a high concentration of organic residues in suspension.

Introduction

European Union (EU) regulations establish the framework and maximum allowed limits for specific pollutants in water.[1–5] Directive (EU) 2020/2184 establishes the parameters to be measured in water intended for human consumption and their parametric values.[1] Directive 2013/39/EU provides a list of priority substances that should be monitored in surface waters and biota.[4] The limits specified in Directive 2013/39/EU are challenging to measure and are in most of cases stricter than those specified in Directive (EU) 2020/2184.

The objective of the methodology described in this application note is to facilitate analysis of sVOCs listed in the aforementioned Directives that are GC-amenable and can be included in a multiresidue method. Certain pollutants were excluded because they require analysis using a specific, rather than generic, method.

For sample preparation, a simple, fast, reliable, and inexpensive extraction procedure is desirable to enhance confidence in results while reducing cost-per-sample and turnaround time. Methodology that uses lower sample volumes compared to traditional extraction methods would be advantageous for sample delivery and handling. In addition, performing analyses at the required sub-ng/L levels is a challenge because of the potential for laboratory cross-contamination. Some sVOCs are persistent and can remain in thoroughly cleaned laboratory supplies. The ideal sample preparation materials should be inexpensive and disposable after use to minimize cross-contamination.

This application note presents a novel sample extraction procedure—low-density microextraction (LDME) based on dispersive liquid-liquid microextraction—which, when coupled with an Agilent 7010 triple quadrupole GC/MS (GC/TQ), enables laboratories to routinely achieve sub-ng/L detection limits for target sVOCs. The method uses less than 20 mL of water sample and only few µL of a non-chlorinated organic solvent.

In the LDME method, a small volume of toluene is added to a disposable screw cap vial containing a low-volume water sample. A mixing vortex is applied to generate multiple micro-drops that accelerate the migration of the sVOCs into the organic phase, creating a nearly instantaneous equilibrium between phases. Selection of the suitable extraction solvent is key to successful analyte extraction and analysis. Toluene was selected as the extraction solvent because it is completely immiscible with water, moderately polar, and has density lower than 1.0, making it ideal for liquid-liquid microextraction of medium- to low-polarity organic compounds in water samples. Unlike high-throughput dispersive liquid/liquid microextraction (DLLME), a dispersant solvent such as methanol or acetone is not necessary for LDME. In addition, toluene supports good chromatographic performance with low boiling (< 140 °C) and melting (< −50 °C) points, and is economical, found in most laboratories, and non-chlorinated. Although it is possible to work with concentration factors of 1:250 solvent to sample volume or higher, a ratio of 1:100 was used to prioritize achieving routine analytical robustness over sensitivity.

For drinking water samples, centrifugation is used to separate the phases after mixing. For samples with a high organic load, such as surface waters, centrifugation following mixing is not recommended due to observed analyte loss.

To make the separation easier, the vial contents are chilled to freezing, while leaving the upper organic layer in the liquid phase so that it can be easily recovered and placed in a vial with a conical insert.

Due to the demanding LODs and LOQs that must be achieved, sVOCs analysis uses triple quadrupole technology. The Agilent 7010 GC/TQ provides the outstanding sensitivity and selectivity needed to reach the desired limits.

This application note presents the linearity, accuracy, repeatability, reproducibility, sensitivity, and carryover performance obtained by validating the LDME GC/TQ method following ISO 17025 guidelines.[6] Interlaboratory proficiency tests were performed on drinking (tap) and surface water samples to compare the results obtained from LDME with Stir Bar Sorptive Extraction (SBSE) and Solid Phase Extraction (SPE). Proficiency testing also studied matrix effects on method results in surface water samples containing a high concentration of organic residue in suspension.

Experimental

Chemicals and standards

The reactants and solvents used included GC/MS-grade toluene, GC-quality acetonitrile, and GC-grade sodium thiosulfate 5-hydrate.

A working solution was prepared in water to a final concentration of 100 g/L of sodium thiosulfate. Certified custom-made standard solutions of all analytes and internal standards (ISTDs) of chlorpyriphos-methyl d_6, atrazine d_5, 4,4'-DDE d_8, benzo(a)anthracene-d_{12}, benzo(a)pyrene-d_{12}, benzo(b)fluoranthene-d_{12}, and chrysene-d_{12} were used to validate and demonstrate method performance.

Sample preparation

LDME is a simple liquid-liquid extraction that uses an organic solvent that is completely immiscible in water, but with enough polarity to extract a wide variety of sVOCs, using a concentration factor between 1:100 to 1:250 depending on the water and toluene volume. The results presented here were obtained using a concentration factor of 1:100. The method was developed as a robust and routine protocol for high-throughput laboratories where numerous and varied water samples are analyzed, including some with a high organic content.

The equipment needed to carry out the LDME includes:

- Vortex
- Circulating cryothermostat down to –30 °C
- Centrifuge
- Centrifuge tubes, 50 mL (Falcon tubes)
- Micropipettes with disposable pipette tips

LDME workflow (Figure 1):

1. Add 15 mL of water sample (taken with a 20 mL micropipette tip) to a 20 mL screw cap vial.
2. Add 30 µL of ISTD mix. The amount of ISTDs in the sample are 0.1 µg/L for pesticides and 0.01 µg/L for polycyclic aromatic hydrocarbons (PAHs).
3. Close the vial and agitate it in vortex for 10 seconds.
4. Add 150 µL of toluene using a disposable micropipette tip and agitate in vortex for 30 seconds.
5. For low-organic-load samples only, centrifuge for 5 minutes at 3,500 rpm.
6. Put the vials in the circulation cryothermostat at –30 °C until the aqueous phase is completely frozen (about 30 min).
7. Recover 70 µL of the upper layer using a disposable pipette tip and pour it into a 2 mL vial with glass insert.

Note: The same procedure is used to analyze calibration samples.

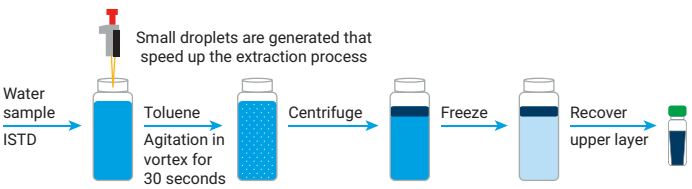

Figure 1. LDME procedure overview.

GC/TQ instrumentation and analysis

GC/TQ analyses were performed using an Agilent 8890 Gas Chromatograph (GC) System coupled to an Agilent 7010 GC/TQ equipped with the high-efficiency ion source (HES). The HES is designed to deliver confident ultra-trace-level analysis. The GC/TQ was operated in dynamic multiple reaction monitoring (dMRM) mode. The MRM conditions were taken from the Agilent Pesticide and Environmental Pollutant MRM Database (part number G9250AA).

Sample introduction used an Agilent 7693 Automatic Liquid Sampler (ALS) with 10 µL syringe (part number 5181-1267), and an ultra-inert split/splitless injection port. Agilent MassHunter Software was used for instrument control and qualitative and quantitative data analysis. The instrument parameters are provided in Table 1.

Table 1. GC/TQ parameters

Parameter	Value
GC System	Agilent 8890 GC System
Column	Agilent HP-5ms Ultra Inert, 30 m × 0.25 m, 0.25 µm (p/n 19091S-433UI)
Carrier Gas	Helium
Retention Time Locking	Chlorpyriphos-methyl locked at 8.143 min
Column Flow	1 mL/min
Liner	Agilent Ultra Inert splitless liner with glass wool (p/n 5190-3163)
Inlet	Inert split/splitless, pulsed splitless during min 1.0
Inlet Temperature	280 °C
Oven Program	100.0 °C (1.0 min), 40 °C/min to 170 °C, 10 °C/min to 310 °C (4 min)
Injection Volume	4 µL (Toluene as solvent)
MS System	Agilent 7010 GC/TQ
MS Source Voltage	–70 eV
Emission Current	100 µA
Source Temperature	280 °C
Quadrupole Temperature	150 °C
Transfer Line Temperature	280 °C
Solvent Delay	3.0 min
Helium Quench Gas	1.5 mL/min
Collision Gas	Nitrogen
Acquisition Mode	dMRM
MRM Parameters	Agilent Pesticide and Environmental Pollutant MRM database (p/n G9250AA)

Results and discussion

Linearity

Mineral water was used as blank matrix to evaluate the method's linear range. Blank and calibration level samples were extracted using the LDME procedure. The amount of the target compounds in the calibration levels are listed in Table 2. The calibration levels were designed to address compounds that have very low parametric values in the EU regulations. Linearity was evaluated by injecting the different calibration levels and calculating the R^2 and accuracy of the residual values (the difference between the predicted response based on the calibration line and the actual measured response of the standard). The allowed values were $R^2 > 0.990$ and accuracy of residuals < 20%, or within 80 to 120% as provided by MassHunter Software (Table 3). Example calibration curves are shown in Figure 2.

Table 2. Target compound concentration per calibration curve level.

Calibration Level	Aldrin (µg/L)	Dieldrin (µg/L)	Endrin (µg/L)	HCB (µg/L)	Heptachlor (µg/L)	Heptachlor epoxide-A (µg/L)	Fipronil (µg/L)	Cadusafos (µg/L)	Rest of Pesticides (µg/L)	PAHs (µg/L)	Pesticides ISTD (µg/L)	PAHs ISTD (µg/L)
Level 1	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0008	0.0012	0.002	0.0002	0.100	0.010
Level 2	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.002	0.003	0.005	0.0005	0.100	0.010
Level 3	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0040	0.0060	0.010	0.0010	0.100	0.010
Level 4	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.010	0.015	0.025	0.0025	0.100	0.010
Level 5	0.015	0.015	0.015	0.015	0.015	0.015	0.020	0.030	0.050	0.005	0.100	0.010
Level 6	0.060	0.060	0.060	0.060	0.060	0.060	0.080	0.120	0.200	0.020	0.100	0.010
Level 7	0.120	0.120	0.120	0.120	0.120	0.120	0.160	0.240	0.400	0.040	0.100	0.010

Table 3. MRMs monitored per compound, average linearity (R^2), and accuracy of residual values obtained during validation.

Name	Primary Transition	Secondary Transition	R^2	0.005 ppb Accuracy (%)	0.010 ppb Accuracy (%)	0.025 ppb Accuracy (%)	0.50 ppb Accuracy (%)	0.20 ppb Accuracy (%)	0.40 ppb Accuracy (%)
4,4'-Dichlorobenzophenone	250 → 139 (10 eV)	250 → 215 (5 eV)	0.999	96	96	94	101	103	99
Aclonifen	264 → 77 (35 eV)	212 → 182 (10 eV)	1.000	96	96	97	103	100	100
Acrinathrin	289 → 93 (10 eV)	247 → 68 (30 eV)	1.000	107	95	97	102	102	99
Alachlor	269 → 188 (5 eV)	237 → 160 (5 eV)	0.999	97	93	95	103	104	98
Aldrin	263 → 228 (20 eV)	255 → 220 (20 eV)	0.996	90	91	105	103	109	95
Ametryn	227 → 212 (10 eV)	227 → 170 (5 eV)	0.997	101	91	100	105	107	96
Atrazine	215 → 138 (15 eV)	215 → 58 (10 eV)	0.999	95	96	98	107	104	98
Azinphos-ethyl	160 → 77 (20 eV)	160 → 132 (0 eV)	0.998	102	95	87	98	96	103
Benalaxyl	266 → 148 (5 eV)	234 → 146 (20 eV)	0.998	104	100	102	115	96	100
Benfluralin	292 → 160 (25 eV)	292 → 264 (5 eV)	1.000	98	94	96	102	101	100
Benzo(a)pyrene	252 → 224 (60 eV)	252 → 250 (45 eV)	0.999	103	91	101	107	103	98
Benzo(b)fluoranthene	252 → 248 (60 eV)	252 → 224 (60 eV)	0.997	99	98	95	104	107	97
Benzo(g,h,i)perylene	276 → 273 (60 eV)	276 → 272 (60 eV)	0.994	99	92	99	116	107	95
Benzo(k)fluoranthene	252 → 250 (45 eV)	252 → 224 (60 eV)	0.997	92	88	99	113	104	97
Bifenthrin	181 → 115 (55 eV)	181 → 165 (25 eV)	1.000	99	98	98	106	101	99
Bromopropylate	341 → 157 (45 eV)	341 → 183 (20 eV)	1.000	100	98	99	106	101	99
Cadusafos	213 → 89 (10 eV)	159 → 97 (15 eV)	0.998	98	96	101	108	104	97
Chlorphenvinfos	323 → 267 (10 eV)	267 → 159 (15 eV)	0.999	98	96	91	96	101	101
Chlorpropham	213 → 127 (10 eV)	213 → 171 (5 eV)	0.998	95	101	101	110	105	96
Chlorpyrifos	314 → 258 (15 eV)	314 → 286 (5 eV)	0.999	94	94	98	105	104	98
Chlorpyrifos-methyl	286 → 93 (20 eV)	286 → 271 (15 eV)	0.998	94	95	99	107	105	97
Cyfluthrin	226 → 206 (15 eV)	163 → 127 (5 eV)	0.999	99	97	101	103	104	98
Cypermethrin	165 → 127 (5 eV)	163 → 127 (5 eV)	1.000	100	95	98	103	102	99

Table 3. MRMs monitored per compound, average linearity (R^2), and accuracy of residual values obtained during validation.

Name	Primary Transition	Secondary Transition	R^2	0.005 ppb Accuracy (%)	0.010 ppb Accuracy (%)	0.025 ppb Accuracy (%)	0.50 ppb Accuracy (%)	0.20 ppb Accuracy (%)	0.40 ppb Accuracy (%)
DDD-o,p'	235 → 165 (20 eV)	235 → 199 (15 eV)	0.999	97	94	97	103	103	98
DDE-o,p'	318 → 176 (55 eV)	318 → 248 (15 eV)	0.999	95	97	98	105	104	98
DDE-p,p'	318 → 248 (15 eV)	318 → 176 (55 eV)	0.999	93	95	99	104	104	98
DDT-o,p'+DDD-p,p'	320 → 237 (3 eV)	235 → 199 (15 eV)	0.999	99	97	98	104	102	99
DDT-p,p'	235 → 199 (15 eV)	235 → 165 (20 eV)	0.999	100	95	93	100	97	102
Deltamethrin	251 → 172 (5 eV)	172 → 93 (10 eV)	0.999	110	96	100	106	104	98
Diazinon	304 → 137 (40 eV)	304 → 179 (15 eV)	0.999	97	95	99	108	104	97
Dieldrin	277 → 241 (5 eV)	262.8 → 227.9 (20 eV)	0.997	96	93	102	110	105	96
Difenoconazol	323 → 265 (15 eV)	265 → 139 (45 eV)	0.998	104	98	104	110	108	99
Endosulfan Alpha	241 → 206 (15 eV)	239 → 204 (15 eV)	0.997	95	97	101	113	105	96
Endosulfan Beta	241 → 206 (25 eV)	239 → 204 (25 eV)	0.998	96	94	103	110	105	96
Endosulfan Sulfate	387 → 289 (5 eV)	270 → 235 (15 eV)	1.000	98	93	99	105	99	100
Endrin	262.8 → 227.9 (20 eV)	242.8 → 173 (30 eV)	1.000	102	100	96	102	98	101
Epoxiconazol	192 → 111 (25 eV)	192 → 138 (10 eV)	1.000	97	97	96	102	100	100
Ethion	231 → 129 (20 eV)	231 → 175 (10 eV)	1.000	100	97	96	101	101	100
Ethofumesate	286 → 161 (20 eV)	286 → 207 (5 eV)	0.999	97	95	97	106	103	98
Etofenprox	163 → 107 (20 eV)	163 → 135 (10 eV)	0.998	98	97	104	109	103	97
Fenazaquin	160 → 117 (20 eV)	160 → 145 (5 eV)	0.999	99	98	99	106	102	98
Fenitrothion	277 → 109 (15 eV)	260 → 125 (10 eV)	1.000	99	97	94	99	102	100
Fenothrin	183 → 115 (22 eV)	183 → 153 (15 eV)	1.000	103	95	101	101	100	100
Fenpropathrin	265 → 210 (10 eV)	264.9 → 89 (30 eV)	1.000	100	97	98	106	101	99
Fenthion	278 → 169 (15 eV)	278 → 125 (15 eV)	0.999	98	96	101	107	102	98
Fenvalerate	225 → 119 (15 eV)	167 → 125 (5 eV)	0.998	104	94	100	108	105	97
Fipronil	367 → 255 (25 eV)	367 → 213 (25 eV)	1.000	100	104	96	98	102	100
Flucythrinate	225 → 119 (15 eV)	199 → 107 (25 eV)	0.999	101	98	102	108	103	97
Fludioxonil	248 → 127 (30 eV)	248 → 154 (20 eV)	1.000	97	96	94	98	101	100
Heptachlor	274 → 239 (15 eV)	272 → 237 (15 eV)	0.994	92	92	107	106	110	94
Heptachlor-epoxide-A	289 → 219 (30 eV)	289 → 253 (10 eV)	0.997	92	94	101	107	106	96
Heptachlor-epoxide-B	353 → 282 (20 eV)	353 → 263 (15 eV)	0.997	95	98	101	108	106	96
Hexachlorobenzene	284 → 214 (30 eV)	284 → 249 (15 eV)	0.997	85	95	109	113	104	98
Hexachlorocyclohexane-alpha	219 → 109 (40 eV)	219 → 183 (5 eV)	0.996	93	95	103	112	107	95
Hexaclorociclohexano-beta	219 → 109 (40 eV)	219 → 183 (5 eV)	0.996	95	95	103	112	106	95
Hexaclorociclohexano-delta	219 → 109 (40 eV)	219 → 183 (5 eV)	0.997	94	95	102	111	106	96
Indene(123,cd)pyrene	276 → 274 (50 eV)	276 → 276.2 (37 eV)	0.995	84	84	101	113	107	95
Isodrin	193 → 157 (20 eV)	263 → 228 (20 eV)	0.996	82	92	103	103	108	96
Kresoxim Methyl	206 → 131 (10 eV)	206 → 116 (5 eV)	1.000	97	94	94	101	102	99
Lambda-cyhalothrin	199 → 161 (5 eV)	197 → 161 (5 eV)	0.999	99	95	99	106	103	98
Lindane-gamma	219 → 109 (40 eV)	219 → 183 (5 eV)	0.997	95	95	105	111	106	96
Malathion	173 → 99 (15 eV)	158 → 125 (10 eV)	0.999	97	99	99	105	104	98
Metazachlor	209 → 132 (15 eV)	209 → 133.2 (10 eV)	0.999	95	93	96	100	101	94
Methidathion	145 → 58 (15 eV)	145 → 85 (5 eV)	1.000	97	99	99	106	101	99
Methiocarb	168 → 91 (30 eV)	168 → 109 (15 eV)	0.998	98	97	100	103	106	97
Methoxychlor	227 → 141 (40 eV)	227 → 169 (25 eV)	0.999	101	97	95	107	100	100
Metolachlor	238 → 133 (30 eV)	238 → 162 (10 eV)	0.997	97	98	102	109	106	96
Metribuzin	198 → 82 (15 eV)	198 → 110 (10 eV)	0.998	94	100	96	111	94	102

Table 3. MRMs monitored per compound, average linearity (R^2), and accuracy of residual values obtained during validation.

Name	Primary Transition	Secondary Transition	R^2	0.005 ppb Accuracy (%)	0.010 ppb Accuracy (%)	0.025 ppb Accuracy (%)	0.50 ppb Accuracy (%)	0.20 ppb Accuracy (%)	0.40 ppb Accuracy (%)
Molinate	187 → 55 (20 eV)	187 → 126 (5 eV)	0.994	94	98	108	117	107	94
Myclobutanil	179 → 90 (30 eV)	179 → 125 (10 eV)	0.999	95	95	97	103	104	98
Oxadiazon	258 → 112 (30 eV)	258 → 175 (5 eV)	0.998	96	94	99	108	104	97
Parathion Ethyl	291 → 91 (25 eV)	291 → 109 (10 eV)	0.999	99	94	90	96	100	101
Parathion Methyl	263 → 79 (30 eV)	263 → 109 (10 eV)	1.000	97	99	97	103	102	99
Pendimethalin	252 → 191 (10 eV)	252 → 162 (10 eV)	1.000	98	94	95	100	100	100
Pentachlorobenzene	250 → 179 (30 eV)	250 → 215 (20 eV)	0.992	71	95	119	118	109	97
Permethrin	183 → 128 (30 eV)	163 → 127 (5 eV)	0.999	100	98	100	107	103	98
Piperonyl Butoxide	176.1 → 103.1 (25 eV)	176 → 131 (15 eV)	0.999	99	97	91	97	98	102
Pirimicarb	238 → 166 (10 eV)	166 → 96 (15 eV)	0.998	95	91	110	110	119	98
Pirimiphos Methyl	290 → 125 (20 eV)	290 → 151 (15 eV)	0.999	99	95	99	105	104	98
Procymidone	283 → 67 (40 eV)	283 → 96 (10 eV)	0.998	95	96	102	107	105	97
Prometryn	241 → 184 (10 eV)	241 → 58 (10 eV)	0.998	100	97	102	104	107	96
Propachlor	211 → 120 (18 eV)	196 → 120 (10 eV)	0.996	94	97	109	115	112	98
Propanil	161 → 99 (30 eV)	217 → 161 (10 eV)	0.996	92	93	102	96	109	96
Propazine	229 → 58 (10 eV)	214 → 172 (10 eV)	0.993	94	91	105	109	110	94
Propyzamide	254 → 226 (15 eV)	173 → 109 (30 eV)	0.997	95	97	102	109	106	96
Pyrazophos	265 → 138 (25 eV)	265 → 210 (10 eV)	1.000	99	95	94	100	102	100
Pyridaben	309 → 132 (40 eV)	309 → 147 (15 eV)	1.000	103	98	98	101	103	99
Pyrimethanil	198 → 118 (35 eV)	198 → 158 (20 eV)	0.999	93	91	97	107	104	98
Quinoxyfen	307 → 237 (20 eV)	307 → 272 (5 eV)	0.998	102	92	96	105	94	103
Quizalafop Ethyl	372 → 244 (25 eV)	372 → 299 (10 eV)	1.000	102	94	97	100	100	100
Simazine	201 → 44 (15 eV)	201 → 138 (10 eV)	0.999	91	93	100	98	104	99
Tebuconazole	250 → 125 (20 eV)	250 → 153 (10 eV)	1.000	95	94	99	102	101	100
Tebufenpyrad	318 → 145 (15 eV)	318 → 131 (15 eV)	1.000	101	98	98	104	100	100
Terbuthylazine	214 → 104 (20 eV)	214 → 132 (10 eV)	0.997	95	93	101	109	106	96
Terbutryn	241 → 170 (15 eV)	226 → 96 (20 eV)	0.999	97	101	99	106	105	97
Tetradifon	354 → 227 (10 eV)	354 → 159 (10 eV)	1.000	98	94	97	104	102	99
Tetramethrin	164 → 77 (25 eV)	164 → 107 (10 eV)	0.999	93	97	90	102	99	101
Tolclofos Methyl	265 → 220 (25 eV)	265 → 93 (25 eV)	0.998	96	96	102	109	105	96
Trifluralin	306 → 264 (5 eV)	290 → 248 (5 eV)	0.999	99	96	100	105	102	99
Vinclozolin	212 → 145 (25 eV)	212 → 172 (15 eV)	0.999	99	94	100	107	104	97

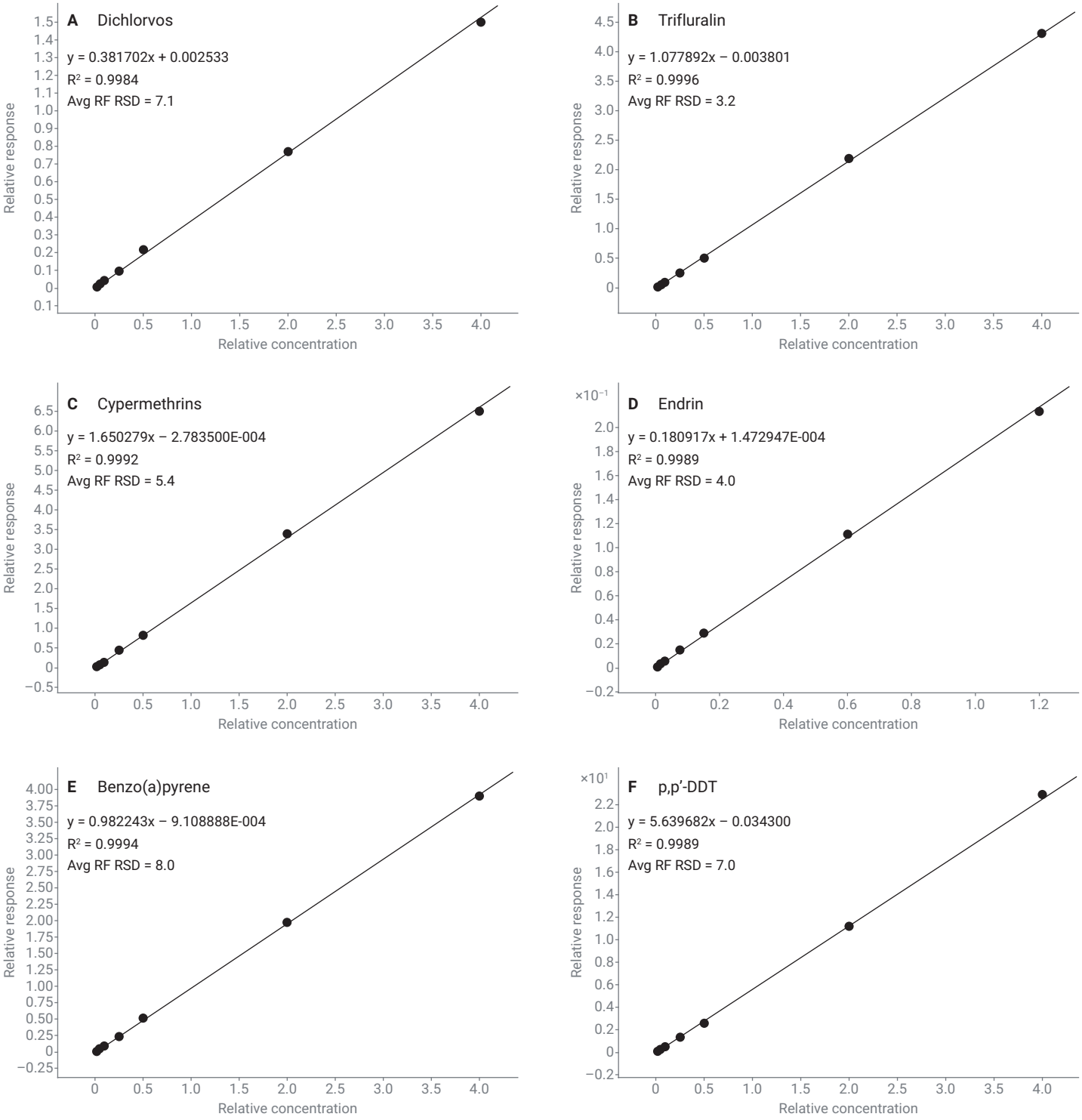

Figure 2. Example calibration curves for dichlorvos, trifluralin, cypermethrins, endrin, benzo(a)pyrene, and p,p'-DDT developed using the LDME GC/TQ method.

Repeatability, reproducibility, and recovery

Repeatability determinations were made by analyzing 10 blank samples spiked at their parametric and LOQ values. Analyses were carried out during the same day and by the same analyst.

Reproducibility was evaluated using data obtained from analyses of more than 150 spiked water samples (mineral, tap, and surface waters) at different known concentration levels, carried out over a year by different analysts.

According to Directive 98/83/EC, the parametric values (PVs) for water intended for human consumption are:

- Benzo(a)pyrene: 0.010 µg/L
- Sum of benzo(b)fluorantheno, benzo(k)fluoranthene, benzo(g,h,i)perylene, and indene(1,2,3-cd)pyrene: 0.10 µg/L
- Aldrin, dieldrin, heptachlor, heptachlor epoxide: 0.03 µg/L
- Rest of pesticides: 0.10 µg/L.

Very good percent recovery (well within the 75 to 125% acceptable range) and precision were obtained for all compounds tested in both the repeatability and reproducibility experiments. Table 4 lists the results per compound.

Table 4. LOD, LOQ, and repeatability and reproducibility results with recovery and precision per compound.

Compound	Linear Range (µg/L)	LOD (µg/L)	LOQ (µg/L)	PV (µg/L)	Repeatability		Reproducibility	
					Recovery (%)	Precision	Recovery (%)	Precision
4,4'-Dichlorobenzophenone	0.002 – 0.40	0.002	0.005	0.10	103.5	11	100.6	12.4
Aclonifen	0.002 – 0.40	0.002	0.005	0.10	100.9	9	96.2	16.3
Acrinathrin	0.002 – 0.40	0.002	0.005	0.10	103.8	10.9	96.3	14.7
Alachlor	0.002 – 0.40	0.002	0.005	0.10	103.9	9.4	97.9	11.7
Aldrin	0.0006 – 0.12	0.0006	0.0015	0.03	115.7	12.9	102.5	10.5
Ametryn	0.002 – 0.40	0.002	0.005	0.10	104.8	10.9	97.1	11.3
Atrazine	0.002 – 0.40	0.002	0.005	0.10	104.8	7.9	95.1	11.6
Azinphos-ethyl	0.002 – 0.40	0.002	0.005	0.10	101.6	11.9	94.6	14.3
Benalaxyl	0.002 – 0.40	0.002	0.005	0.10	105.7	11.4	90.7	11.4
Benfluralin	0.002 – 0.40	0.002	0.005	0.10	103.8	7.9	97	15.3
Benzo(a)pyrene	0.001 – 0.040	0.001	0.0025	0.01	111.8	9.9	102.1	10.7
Benzo(b)fluoranthene	0.001 – 0.040	0.001	0.0025	0.10	108.1	9.8	99.9	11.4
Benzo(g,h,i)perylene	0.001 – 0.040	0.001	0.0025	0.10	114.1	8.3	100.4	12.7
Benzo(k)fluoranthene	0.001 – 0.040	0.001	0.0025	0.10	110.9	9.9	98.1	11.9
Bifenthrin	0.002 – 0.40	0.002	0.005	0.10	99.2	10.1	93.3	11.8
Bromopropylate	0.002 – 0.40	0.002	0.005	0.10	106.7	16	94	11.6
Cadusafos	0.002 – 0.40	0.002	0.005	0.10	102.8	8.3	101.3	14
Chlorphenvinfos	0.002 – 0.40	0.002	0.005	0.10	97.6	9.6	97.5	15.2
Chlorpropham	0.002 – 0.40	0.002	0.005	0.10	116.2	12.3	105.5	13.3
Chlorpyrifos	0.002 – 0.40	0.002	0.005	0.10	105.5	9.2	96.8	10.4
Chlorpyrifos-methyl	0.002 – 0.40	0.002	0.005	0.10	106	7.9	95.4	9.5
Cyfluthrin	0.002 – 0.40	0.002	0.005	0.10	105	11.2	93	14.3
Cypermethrin	0.002 – 0.40	0.002	0.005	0.10	108.9	12.6	96	13.5
DDD-o,p'	0.002 – 0.40	0.002	0.005	0.10	101	8.6	96.6	8.7
DDD-p,p'	0.002 – 0.40	0.002	0.005	0.10	102.4	8	96	8.5
DDE-o,p'	0.002 – 0.40	0.002	0.005	0.10	106	7.9	98.4	9.4
DDE-p,p'	0.002 – 0.40	0.002	0.005	0.10	105.9	8.5	98.5	9.2
DDT-o,p'	0.002 – 0.40	0.002	0.005	0.10	106.1	11.8	100.8	15.9
DDT-p,p'	0.002 – 0.40	0.002	0.005	0.10	115	20.2	94.7	15.6
Deltamethrin	0.002 – 0.40	0.002	0.005	0.10	116.8	20.4	105.4	15.7

Table 4. LOD, LOQ, and repeatability and reproducibility results with recovery and precision per compound.

Compound	Linear Range (µg/L)	LOD (µg/L)	LOQ (µg/L)	PV (µg/L)	Repeatability		Reproducibility	
					Recovery (%)	Precision	Recovery (%)	Precision
Diazinon	0.002 – 0.40	0.002	0.005	0.10	104.9	9.2	96.9	11
Dieldrin	0.0006 – 0.12	0.0006	0.0015	0.03	108.5	8.5	99.8	10.1
Difenoconazol	0.002 – 0.40	0.002	0.005	0.10	116.3	19.3	99.1	14.4
Endosulfan Alpha	0.002 – 0.40	0.002	0.005	0.10	107.8	8	99.1	10.1
Endosulfan Beta	0.002 – 0.40	0.002	0.005	0.10	106.8	8.7	98.6	10.5
Endosulfan Sulfate	0.002 – 0.40	0.002	0.005	0.10	104.4	9.6	95.8	10.8
Endrin	0.0006 – 0.12	0.0006	0.0015	0.10	107.4	10.1	97.1	18.6
Epoxiconazol	0.002 – 0.40	0.002	0.005	0.10	96.3	8.5	97.1	16.7
Ethion	0.002 – 0.40	0.002	0.005	0.10	108.7	17.3	99	13.1
Ethofumesate	0.002 – 0.40	0.002	0.005	0.10	106.2	10.6	99.7	12.3
Etofenprox	0.002 – 0.40	0.002	0.005	0.10	107.3	10.3	94.9	11.2
Fenazaquin	0.002 – 0.40	0.002	0.005	0.10	113.3	22.1	99.9	13.5
Fenitrothion	0.002 – 0.40	0.002	0.005	0.10	101.2	8.8	98.6	14.7
Fenothrin	0.002 – 0.40	0.002	0.005	0.10	102	10.4	98.5	17.9
Fenpropathrin	0.002 – 0.40	0.002	0.005	0.10	110.5	17.7	100.5	13.1
Fenthion	0.002 – 0.40	0.002	0.005	0.10	104.6	9.3	98.3	11.6
Fenvalerate+esfenvalerate	0.002 – 0.40	0.002	0.005	0.10	114.7	17.5	102.1	11.5
Fipronil	0.0006 – 0.12	0.0006	0.0015	0.10	111.3	21.8	106.7	17.2
Flucythrinate	0.002 – 0.40	0.002	0.005	0.10	93.2	18.8	89.8	17.3
Fludioxonil	0.002 – 0.40	0.002	0.005	0.10	100.1	10.1	95.5	14
Heptachlor	0.0003 – 0.12	0.0003	0.0006	0.03	117.8	11.2	101.2	13.6
Heptachlor-epoxide-A	0.0006 – 0.12	0.0006	0.0015	0.03	106.9	9.1	99.7	10.8
Heptachlor-epoxide-B	0.0006 – 0.12	0.0006	0.0015	0.03	108.8	9.4	100.2	10.3
Hexachlorobenzene	0.0006 – 0.12	0.0006	0.0015	0.10	120.1	11.6	101.8	11.4
Hexachlorocyclohexane-a	0.002 – 0.40	0.002	0.005	0.10	112.4	7.4	101.7	10
Hexaclorociclohexano-b	0.002 – 0.40	0.002	0.005	0.10	111.8	9.1	102.3	9.5
Hexaclorociclohexano-d	0.002 – 0.40	0.002	0.005	0.10	111.6	8.9	101.3	9.7
Indene(123,cd)pyrene	0.001 – 0.040	0.001	0.0025	0.10	114.4	7.4	96.2	12.2
Isodrin	0.0006 – 0.12	0.0006	0.0015	0.10	111.4	9.2	96.3	10.8
Kresoxim Methyl	0.002 – 0.40	0.002	0.005	0.10	109.6	18.2	103.9	15.1
Lambda-cyhalothrin	0.002 – 0.40	0.002	0.005	0.10	111.2	18.8	99.3	13
Lindane-gamma	0.002 – 0.40	0.002	0.005	0.10	111.8	7.3	103.9	11.5
Malathion	0.002 – 0.40	0.002	0.005	0.10	104.7	9.5	97.8	11.4
Metazachlor	0.002 – 0.40	0.002	0.005	0.10	102.9	7.5	98.6	13.2
Methidathion	0.002 – 0.40	0.002	0.005	0.10	103.9	8.9	98.5	12.9
Methiocarb	0.002 – 0.40	0.002	0.005	0.10	110.7	16.4	92.4	14.7
Methoxychlor	0.002 – 0.40	0.002	0.005	0.10	109.9	15.7	89.4	15.4
Metolachlor	0.002 – 0.40	0.002	0.005	0.10	106.3	9.3	98.5	11.8
Metribuzin	0.002 – 0.40	0.002	0.005	0.10	104.7	9.8	95.7	14.7
Molinate	0.002 – 0.40	0.002	0.005	0.10	113.8	8.1	102.7	12.7
Myclobutanil	0.002 – 0.40	0.002	0.005	0.10	100.6	9.1	97.7	13.6
Oxadiazon	0.002 – 0.40	0.002	0.005	0.10	103.6	8.7	94.8	10.3
Parathion Ethyl	0.002 – 0.40	0.002	0.005	0.10	99.2	8.3	99.2	17.8
Parathion Methyl	0.002 – 0.40	0.002	0.005	0.10	102.3	8.7	99.7	13.9

Table 4. LOD, LOQ, and repeatability and reproducibility results with recovery and precision per compound.

Compound	Linear Range (µg/L)	LOD (µg/L)	LOQ (µg/L)	PV (µg/L)	Repeatability		Reproducibility	
					Recovery (%)	Precision	Recovery (%)	Precision
Pendimethalin	0.002 – 0.40	0.002	0.005	0.10	98.2	8.8	97.3	15.9
Pentachlorobenzene	0.002 – 0.40	0.002	0.005	0.10	116.4	6.9	101.7	13.3
Permethrin	0.002 – 0.40	0.002	0.005	0.10	105.2	9.2	93.4	13.5
Piperonyl Butoxide	0.002 – 0.40	0.002	0.005	0.10	110	18	97.3	13.6
Pirimicarb	0.002 – 0.40	0.002	0.005	0.10	115	13.4	100.4	13.2
Pirimiphos Methyl	0.002 – 0.40	0.002	0.005	0.10	103.4	8.8	96	9.8
Procymidone	0.002 – 0.40	0.002	0.005	0.10	103.9	8.6	94.6	10.3
Prometryn	0.002 – 0.40	0.002	0.005	0.10	104.5	9.8	95	10.8
Propachlor	0.002 – 0.40	0.002	0.005	0.10	114.2	7.4	103.4	11.8
Propanil	0.002 – 0.40	0.002	0.005	0.10	108.7	9.4	98	13.2
Propazine	0.002 – 0.40	0.002	0.005	0.10	110.2	9.7	99.6	11.7
Propyzamide	0.002 – 0.40	0.002	0.005	0.10	108.6	8.1	103.6	11.9
Pyrazophos	0.002 – 0.40	0.002	0.005	0.10	99.5	11.2	94.5	14.5
Pyridaben	0.002 – 0.40	0.002	0.005	0.10	97	8.1	95.4	15.2
Pyrimethanil	0.002 – 0.40	0.002	0.005	0.10	117.4	16.3	102.7	12
Quinoxyfen	0.002 – 0.40	0.002	0.005	0.10	99.8	8.7	93.5	11.1
Quizalafop Ethyl	0.002 – 0.40	0.002	0.005	0.10	114	21.9	98.8	13.1
Simazine	0.002 – 0.40	0.002	0.005	0.10	103.2	15	98.3	18.9
Tebuconazole	0.002 – 0.40	0.002	0.005	0.10	109.3	17.9	96.4	15.7
Tebufenpyrad	0.002 – 0.40	0.002	0.005	0.10	109.5	17.7	101.3	13
Terbuthylazine	0.002 – 0.40	0.002	0.005	0.10	109	9.5	101.5	11.4
Terbutryn	0.002 – 0.40	0.002	0.005	0.10	103.7	7.1	97.7	11.5
Tetradifon	0.002 – 0.40	0.002	0.005	0.10	102.7	7	92.4	10.5
Tetramethrin	0.002 – 0.40	0.002	0.005	0.10	99.3	10.8	93.8	15.3
Tolclofos Methyl	0.002 – 0.40	0.002	0.005	0.10	106	5.8	94	9.7
Trifluralin	0.002 – 0.40	0.002	0.005	0.10	106.1	7	101.1	12.6
Vinclozolin	0.002 – 0.40	0.002	0.005	0.10	114.1	14.9	103	11.9

Sensitivity

The LOQ and LOD for all compounds evaluated fell in the sub-ng/L region, and well below the specified PVs (Table 4). The MRM chromatograms for selected sVOCs measured at sub-ng/L levels are shown in Figure 3.

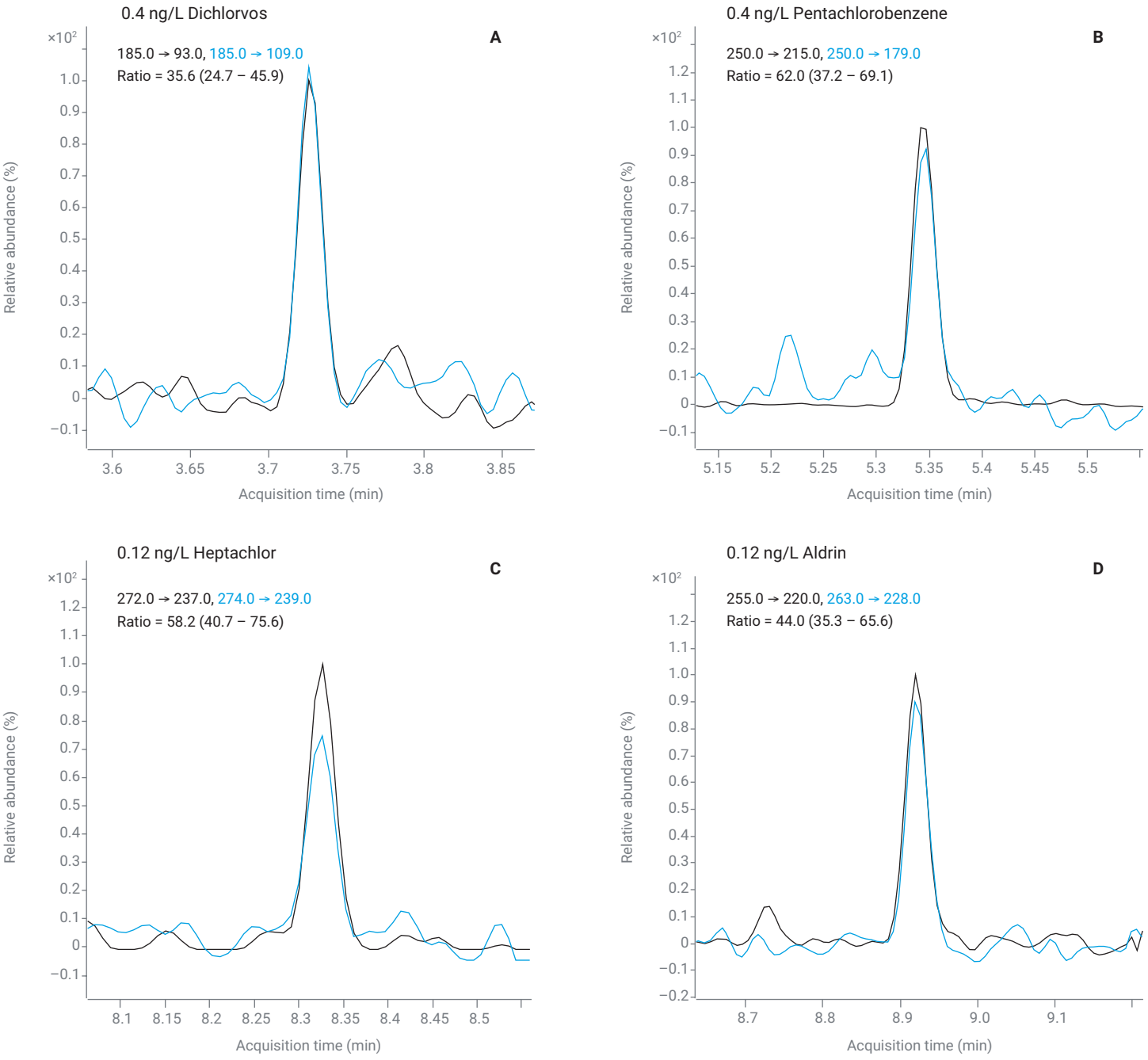

Figure 3A-D. Example MRM chromatograms for selected sVOCs measured at sub-ng/L levels using the LDME GC/TQ method.

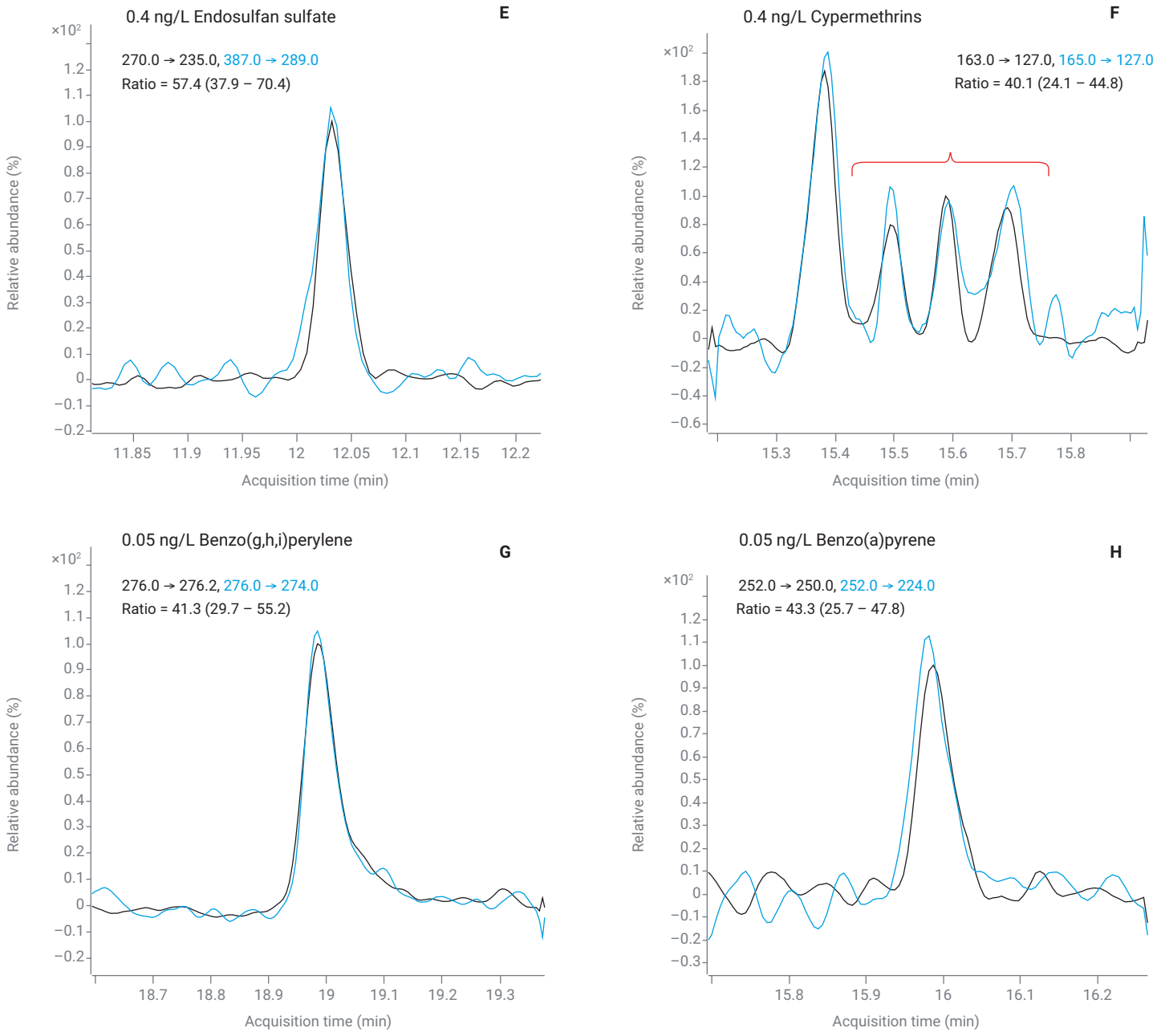

Figure 3E-H. Example MRM chromatograms for selected sVOCs measured at sub-ng/L levels using the LDME GC/TQ method. Note that cypermethrins are composed of four isomers, while the chromatogram showed just three peaks, as indicated by the red bracket.

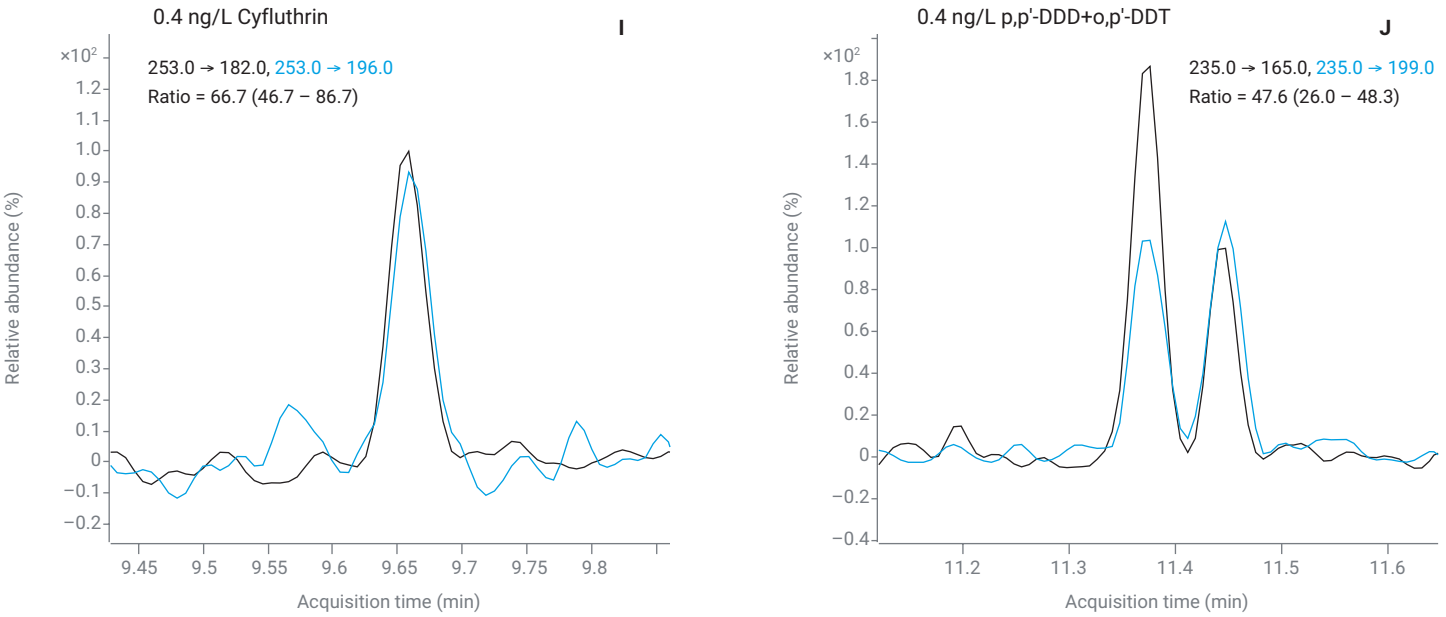

Figure 3I-J. Example MRM chromatograms for selected sVOCs measured at sub-ng/L levels using the LDME GC/TQ method.

Carryover

When working at sub-ng/L measurement levels, all means of cross-contamination must be minimized. The use of disposable materials throughout sample preparation, together with the low carryover of Agilent injection ports, minimize the chance of false positives. To evaluate instrumental carryover for routine analysis using the LDME GC/TQ method, a blank sample was injected after the most concentrated calibration level. The blank was accepted as a true blank if the peak area of the target compounds was less than 1/3 of the peak area obtained in the MRM chromatogram at the LOD in the same sequence. The last eluting PAHs are the most persistent sVOCs present in the scope of the LDME GC/TQ method.

The carryover measured for the most problematic compound (last eluting benzo(g,h,i)perylene) was 0.14%. The peak area measured for benzo(g,h,i)perylene in the blank following injection of the calibration samples was 3% of the area obtained for the LOQ, much lower than the 30% established as the maximum acceptable carryover value (Figure 4).

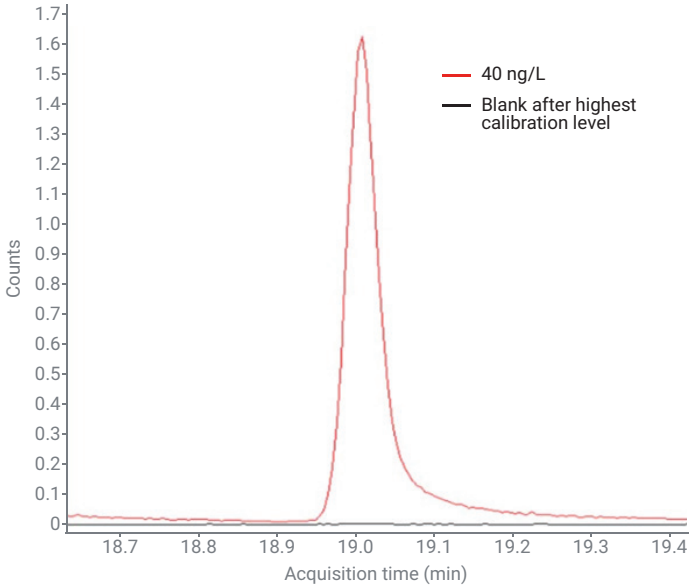

Figure 4. Overlaid MRM chromatograms of the benzo(g,h,i)perylene quantifier transition at the highest calibration level (40 ng/L, red trace) and the following blank injection (black trace).

Proficiency tests

To ensure the quality and accuracy of the results obtained from the LDME GC/TQ method, interlaboratory proficiency tests using drinking and surface water samples were performed with the assistance of IELAB and BIPEA. The interlaboratory results compared three different extraction procedures: LDME, Stir Bar Sorptive Extraction (SBSE), and Solid Phase Extraction (SPE). SBSE and SPE were compared to LDME because they are well recognized and commonly used techniques. Z-scores were calculated to determine how much the LDME method deviated from the mean results obtained using the other extraction methods. IELAB and BIPEA carried out the studies across numerous participating laboratories. The studies were performed over an extended period during 2017 and 2018 by many different analysts.

Tables 5 and 6 present the results from the method comparisons, as well as comparisons with the reference values. The results obtained in the proficiency tests were extremely good. The Z-scores were below two for all compounds evaluated in all proficiency tests, and 86% of Z-score values were below one. It is important to note that LDME is a faster and less expensive procedure than other procedures such as SBSE or SPE.

Table 5. "IELAB-MAY-2017-drinking water" interlaboratory comparison of quantification results obtained from drinking water samples when using LDME, SBDE, or SPE.

Compound	Reference Value (µg/L)	LDME (µg/L)	SBDE (µg/L)	SPE (µg/L)	Z-score LDME	Number of Participant Labs
Endosulfan Alpha	0.13	0.16	0.18	–	0.74	32
Heptachlor	0.068	0.065	0.040	–	−0.19	30
Indene(1,2,3-c,d)pyrene	0.069	0.068	–	0.066	−0.05	32
Propazine	0.089	0.074	0.082	–	−0.67	25
Terbuthylazine	0.21	0.23	0.23	–	0.38	31
Benzo(g,h,i)perylene	0.091	0.074	–	0.078	−0.74	36

Table 6. "BIPEA. Surface water. Round June-2018" interlaboratory comparison of quantification results obtained from surface water samples when using LDME, SBDE, or SPE.

Compound	Reference Value (µg/L)	LDME (µg/L)	SBDE (µg/L)	SPE (µg/L)	Z-score LDME	Number of Participant Labs
Ametryn	0.044	0.041	0.061	–	−0.23	18
Atrazine	0.067	0.063	0.067	–	−0.18	27
Cyanazin	0.079	0.070	0.075	–	−0.39	20
Prometryn	0.053	0.047	0.054	–	−0.38	19
Propazyne	0.057	0.052	0.052	–	−0.31	22
Simazine	0.078	0.074	0.091	–	−0.18	27
Terbutryn	0.041	0.037	0.047	–	−0.26	23
Terbuthylazine	0.070	0.065	0.074	–	−0.25	27
Metazachlor	0.064	0.056	0.058	–	−0.41	24
Metolachlor	0.069	0.060	0.079	–	−0.43	23
Chlortoluron	0.076	0.080	0.117	–	0.14	24
Benzo(a)pyrene	0.012	0.010	–	0.0082	−0.59	29
Benzo(b)fluoranthene	0.013	0.011	–	0.0097	−0.68	29
Benzo(g,h,i)perylene	0.017	0.014	–	0.017	−0.64	30
Benzo(k)fluoranthene	0.012	0.010	–	0.0085	−0.62	28
Indeno(1,2,3-cd)pyrene	0.015	0.012	–	0.013	−0.74	30

Matrix effects

The BIPEA proficiency tests measured surface water samples which contained a high amount of organic residue in suspension. When an extractant solvent with density greater than water was used to extract these samples, all the organic residue was deposited at the bottom of the vial after centrifugation, interfering with microdrop formation. The recoveries of chlorinated pesticides present in the sample with and without centrifugation were evaluated.

As shown in Table 7, recovery of the 16 organochlorinated pesticides tested in surface water samples dropped significantly with centrifugation. For example, the recovery of DDTs and their metabolites was less than 30% with centrifugation, while recoveries were close to 100% without centrifugation. Based on these results, the centrifugation step should be eliminated when analyzing water samples with high organic content.

Table 7. BIPEA comparison of recoveries in surface water samples with and without centrifugation.

Compound	Reference Value (µg/L)	No Centrifugation		With Centrifugation	
		LDME (µg/L)	Z-score LDME	LDME (µg/L)	Z-score LDME
2.4-DDD	0.060	0.074	0.75	0.023	–2.06
2,4-DDE	0.050	0.055	0.30	0.015	–2.33
2,4-DDT	0.058	0.074	0.96	0.016	–2.47
4,4-DDD	0.053	0.064	0.65	0.016	–2.31
4,4-DDE	0.055	0.061	0.37	0.014	–2.48
4,4-DDT	0.054	0.054	–0.13	0.014	–2.35
Aldrin	0.060	0.058	–0.13	0.019	–2.27
Dieldrin	0.073	0.086	0.58	0.051	–1.00
Isodrin	0.045	0.045	0.020	0.015	–2.22
Endosulfan Alpha	0.058	0.062	0.25	0.041	–0.98
Heptachlor	0.055	0.038	–1.0	0.016	–2.35
Heptachlor Epoxide A	0.056	0.068	0.7	0.048	–0.47
Heptachlor Epoxide B	0.057	0.067	0.63	0.042	–0.88
Trifluralin	0.053	0.053	–0.04	0.034	–1.19
Chlorpyriphos Ethyl	0.12	0.15	0.69	0.088	–0.87
Pentachlorobenzene	0.048	0.052	0.27	0.021	–1.88
Hexachlorobenzene	0.044	0.046	0.19	0.015	–2.21

Conclusion

LDME is a rapid, inexpensive, simple, reliable, and robust alternative to traditional water sample preparation techniques such as SBSE and SPE. The method can be conveniently carried out using supplies, solvents, and other materials already present in most laboratories, and therefore does not require a significant investment in additional equipment. All the supplies used, including the extraction vials, pipette tips, 2 mL vials, and glass inserts, are economical to purchase and disposable to avoid carryover contamination. Toluene, the extraction solvent, behaves well as a chromatographic solvent for subsequent GC/TQ analysis.

Freezing the water layer following liquid-liquid separation simplifies the collection of the organic layer. The extraction, preconcentration, and injection of the sample are directly carried out without evaporating the solvent, which can result in the loss of the most volatile compounds. For surface waters with high organic content, the centrifugation step can be omitted without filtering, and recoveries close to 100% can still be obtained.

In addition, because chlorinated solvents are not used in LDME, the method reduces the need to manage chlorinated residues. The volume of water samples and organic solvent is low. This results in lower costs, including delivery costs, and less pollutant emission into the environment.

The performance of the LDME GC/TQ method enables measurement of target sVOCs at the challenging levels specified in Directives (EU) 2020/2184 and 98/83/EC for water intended for human consumption, and Directive 2013/39/EU for surface waters. LDME GC/TQ method performance was validated according to ISO 17025. For all evaluated compounds, linearity was excellent with R^2 greater than 0.99, residuals accuracy was < 20%, and LODs and LOQs were in the sub-ng/L region. Very good percent recoveries were obtained for all compounds tested in both the repeatability experiments (n = 10 spiked blanks analyzed in one day by the same analyst) and reproducibility experiments (n = 150 spiked real-world samples, analyzed over one year by different analysts). Carryover measured for the most problematic last eluting benzo(g,h,i)perylene) was 0.14%, much lower than the 30% maximum the acceptable carryover value.

Proficiency tests using interlaboratory comparisons of the results obtained for drinking water and surface waters demonstrated that the faster and less expensive LDME coupled with GC/TQ detection provides results comparable to the SBSE and SPE methods. Following the 2018 method validation, the robustness of the interlaboratory results have continued, even in high-organic-load matrices not centrifuged or filtered before extraction and analysis.

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