Determination of SVOCs in water samples using the Bruker µDROP™ method for the EVOQ™ GC-TQ MS/MS system

Fast, simple and ultra-sensitive determination of semi-volatile organic compounds in water samples as per European Directives using the Bruker EVOQ GC-TQ Premium MS/MS Triple Quadrupole system with Bruker µDROP method

Abstract

Bruker µDROP is a unique, innovative, simple, economical, and ultra-sensitive method developed for the analysis of a large number of semi-volatile organic compounds (SVOCs) in water samples using the Bruker EVOQ gas chromatography triple quadrupole mass spectrometry system.

The µDROP is a comprehensive solution for the analysis of SVOCs, from extraction through to the final results report. It uses a single injection method and is compliant with the most stringent analytical requirements of current European regulations on water testing.

Authors: Diego Martín-Ortiz, Javier López, Miguel Ángel Pérez.
Bruker Applications Development Laboratory, Madrid, Spain

The methodology has been validated in various water matrices (drinking water, river water and sea water, among others) on the basis of the analytical parameters described in European Directive 2013/39/EU on priority substances in the field of water policy.

Further, the method has been validated in line with the requirements of ISO standard 17025, achieving sub-ppt/ppt (ng/L) detection levels, very good linearity, and excellent reproducibility for all compounds and water matrices under study. The method is ready for implementation in environmental or public health laboratories for the routine quality control of surface water and water for human consumption.

Introduction

Semi-volatile organic compounds (SVOCs) are a large group of moderate volatility substances with very different chemical properties and characteristics. SVOCs include pesticides (organochlorine, organophosphorous, nitrogen-based), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), chloroalkanes, phthalates, phenols, dioxins, and organotin compounds.

Many SVOCs are known to be environmental pollutants, some of which are persistent, remaining in the environment for long periods of time. Currently, the control of organic pollutants in water (drinking, surface and groundwater) is a necessity, as these present a serious threat to aquatic life and a risk of biodiversity loss. The accumulation of such pollutants in the ecosystem poses a consequent threat to human health in general.

Assessment of water quality has therefore been given greater consideration by the environmental authorities in many countries around the world in recent years, as evidenced by adjustments to water quality control legislation to include new regulations featuring extended lists of controlled substances and increasingly stringent quality criteria and detection limits.

Implemented in August 2013, Directive 2013/39/EU [1] forms part of the EU Water Framework Directive [2] and establishes the criteria for the control and assessment of surface water conditions. It amends Directives 2000/60/EC and 2008/105/EC, and defines an extensive list of priority substances to be monitored within the EU, as well as a watch list of substances for monitoring. As a result of this current directive, Environmental Quality Standards (EQS) have been established, with pollutant concentration values expressed as an annual average (AA) and as a maximum allowable concentration (MAC).

Moreover, the recent Directive 2015/1787/EU [3], amending 98/83/EC, establishes the analytical criteria for the quality of water for human consumption.

Earlier regulations established low (sub-ppt/ppt) detection limits, requiring exceptionally sensitive methods for adequate ultratrace analysis. Additionally, any developed method must be sufficiently robust for implementation in process laboratories and for subsequent validation in line with established quality standards (ISO 17025), such that an independent entity can verify that the entire process is compliant with said quality criteria. This is usually referred to as the accreditation process.

In this scenario, the preceding extraction and/or concentration stage is of crucial importance in meeting the required detection limits. This is also usually the bottleneck of the full analytical process, which has traditionally been lengthy, laborious, expensive, and environmentally detrimental.

Commonly used sample preparation techniques such as liquid-liquid extraction (LLE) or solid phase extraction (SPE) are slow and laborious and require large quantities of solvents and samples (>1L). SPE cartridges are single-use, adding to the workflow expense. Furthermore, both techniques create problems in the management of generated wastes.

Various microextraction techniques were subsequently developed. Solid phase microextraction (SPME) was developed in 1990 by Janusz Pawliszyn et al. [4]. Based on the same principle as SPME, Pat Sandra et al. [5] developed the Stir Bar Sorptive Extraction (SBSE) technique in 1999. Both microextraction techniques were an improvement on the standard techniques, but some limitations persist with regard to cost and routine application.

Bruker µDROP is an innovative, miniaturized, and ultra-sensitive method for routine water analysis applications. Further, the method is rapid, low-cost, and environmentally friendly, compliant with current green chemistry recommendations. It is based on the principles of Dispersive Liquid-Liquid Microextraction (DLLME), developed in 2006 by M. Rezaee et al. [6]. This novel microextraction technique involves three liquid phases: a water-immiscible solvent (extractant), a water-miscible solvent (dispersant), and water (sample).

Using this technique, a mist of fine microdroplets of an extractant is dispersed into the aqueous phase, resulting in an immediate extraction of analytes. Subsequently, the fine
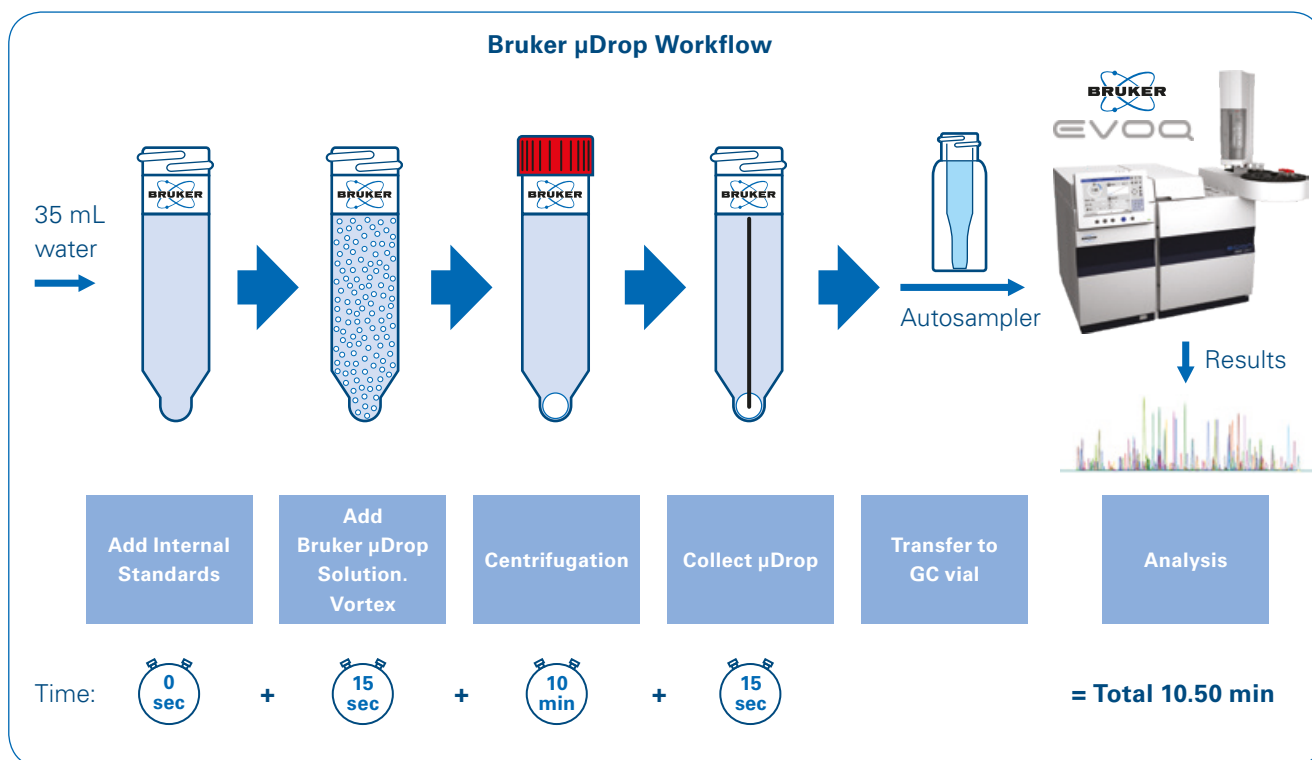
Bruker µDrop Workflow

35 mL water → Autosampler → Results

| Add Internal Standards | Add Bruker µDrop Solution. Vortex | Centrifugation | Collect µDrop | Transfer to GC vial | Analysis |

Time: 0 sec + 15 sec + 10 min + 15 sec = **Total 10.50 min**

Figure 1. Bruker µDROP workflow for sample preparation. For a detailed description of the sample preparation process, please refer to the following document: Instructions for Use: Bruker µDROP Solvent Extraction Mixture #1.

microdroplets become a single microdroplet containing all of the extracted analytes via centrifugation. High recovery and enrichment factors are achieved using this method. A comparison could be drawn with the process that occurs in electrospray ionization (ESI), where a mist of microdroplets is produced by the application of an electric field, while dispersive microextraction creates this effect by chemical means through the dispersant.

Following their development, dispersive microextraction techniques have garnered increasing attention within the analytical world. Extensive literature on the subject has been published[7] and these techniques are applied as extraction methods in various instrumental methodologies. However, in the majority of cases, they have been applied to a small number of analytes or to a single compound class.

The Bruker µDROP method is the result of a proprietary development that allows for the simultaneous extraction of a large number of analytes, from very different chemical families. All SVOCs amenable to GC-MS may be determined in a single injection, meeting or exceeding the requirements of the most stringent environmental water analysis regulations.

Experimental

Sample Preparation: Bruker µDROP Method

The workflow for sample preparation using the Bruker µDROP method is shown in Figure 1. Using a 40-tube centrifuge, up to 40 samples can be prepared in an approximate total time of 10 minutes. After this stage, all analysis is automatic and unattended, with vials being placed in the gas chromatograph autosampler.

The solutions were prepared using individual standards supplied by AccuStandard, Inc (New Haven, CT, USA). Deuterated internal standards were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). All analyzed compounds are described in Table 4.

The general laboratory materials and Bruker consumables used for sample preparation are detailed in Table 1. Instrument conditions are summarized in Table 2.

Analytical Method

A total of 59 semi-volatile compounds included under Directive 2013/39/EU were analyzed. Three internal standards were used for quantification: DDE-4,4´-d8, Terbutryn-d5 and Benzo(a)pyrene-d12.

The acquisition method (Figure 2) was created quickly and easily using the intuitive method editor built into the acquisition software.

The Compound Based Scanning (CBS) software function automatically calculated the scan time for each compound in dynamic windows, optimizing the number of points per peak for precise quantification (Figure 3).

In the absence of time segments, each compound was assigned transitions, so that any modifications to the acquisition were automatically edited in the table of compounds, avoiding manual editing and duplication of work.

Bruker TASQ™ 1.4 was used for sample processing and statistical calculations of quality parameters. With this software, results could be rapidly and comprehensively reviewed (Figure 4), as could the statistical calculations for parameters included in the validation study.

Figure 5 shows the total ion chromatogram (TIC) of a river water sample spiked with 10 ppt of the 62 compounds analyzed. The analyzed compounds elute between 7.5 and 22.5 minutes. Each compound presents at least two transitions (MRM), and the scan time is optimized to obtain at least 12 points across each chromatographic peak, in support of meeting the validation criteria.

Laboratory Materials
Bruker µDROP™ Solvent Extraction Mixture #1 (p/n: 1845184)
Bruker µDROP™ Centrifuge Tubes Kit (p/n: 1850435)
Centrifuge: Non-refrigerated, minimum speed: 3,000-4,000 rpm
Automatic pipettes for liquid handling
2 mL Ultra GCMS vials, screw top wide opening, amber glass with ultra GCMS septa (p/n: 392612016)
200 µL insert, silanized, conical polymer spring (p/n: 392611595)

Table 1: Laboratory materials and Bruker consumables for sample preparation

Mass Spectrometer	Bruker EVOQ GC-TQ MS/MS system
	MS Conditions
Ionization	EI, 70 eV
Emission Current	40 µA
Active Focusing Q0	135 °C with Helium
Transfer Line Temperature	280 °C
Source Temperature	280°C
CID Gas	Ar, 2.0 mtorr
Detector Mode	EDR

Gas Chromatograph	Bruker 436 GC
	GC Conditions
Injector	PTV 1079 with programmable temperature
Injection Mode	LVI with solvent vent step
Injector Insert	Siltek 3.4 mm ID Frit gooseneck (p/n: RT217092145)
GC Oven Temperature	Temperature ramp up to 310 °C
GC Column	Bruker BR-5ms, 30 m x 0.25mm, 0.25 micron (p/n: BR86377)
Carrier Gas	Helium, 1 mL/min constant flow
Total Run Time	29 min
Autosampler	Bruker 8400 autosampler
Software	Bruker Hystar 4.1/TASQ processing software

Table 2: Mass Spectrometry Method Conditions

	Name	Retention Time	RT Window	CAS Number	Retention Index	Scan Type	Scan Time (ms)	Polarity
1	Pentachlorobenzene	8.35	0.40	608-93-5	0	MRM	500.0	Positive
2	Trifluralin	9.38	0.40	1582-09-8	0	MRM	500.0	Positive
3	a-HCH	9.85	0.40		0	MRM	83.3	Positive
4	Hexachlorobenzene	9.95	0.40	118-74-1	0	MRM	83.3	Positive
5	Simazine	10.09	0.40	122-34-9	0	MRM	83.3	Positive
6	Atrazine	10.17	0.40	1912-24-9	0	MRM	83.3	Positive
7	b-HCH	10.25	0.40	319-85-7	0	MRM	83.3	Positive
8	Pentachlorophenol	10.33	0.40	87-86-5	0	MRM	83.3	Positive
9	Lindane	10.40	0.40	58-89-9	0	MRM	83.3	Positive
10	Antraceno	10.80	0.40	120-12-7	0	MRM	250.0	Positive
11	d-HCH	10.85	0.40	319-86-8	0	MRM	250.0	Positive
12	Endosulfan I	11.20	0.40	959-98-8	0	MRM	250.0	Positive
13	Alachlor	11.46	0.40	15972-60-8	0	MRM	125.0	Positive
14	Heptachlor	11.67	0.40	76-44-8	0	MRM	125.0	Positive
15	Terbutryn D5	11.81	0.40		0	MRM	125.0	Positive
16	Terbutryn	11.85	0.40	886-50-0	0	MRM	125.0	Positive
17	Chlorpyrifos	12.15	0.40	2921-88-2	0	MRM	166.7	Positive
18	Aldrin	12.32	0.40	309-00-2	0	MRM	166.7	Positive
19	Dicofol	12.44	0.40	115-32-2	0	MRM	166.7	Positive
20	Dieldrin	12.84	0.40	60-57-1	0	MRM	83.3	Positive
21	Chlorphenvinfos	12.90	0.40	470-90-6	0	MRM	83.3	Positive
22	Heptachlor epoxide A	12.97	0.40	28044-83-9	0	MRM	83.3	Positive
23	Cibutrina	12.98	0.40	28159-98-0	0	MRM	83.3	Positive
24	Heptachlor epoxide B	12.99	0.40	1024-57-3	0	MRM	83.3	Positive
25	Fluoranteno	13.16	0.40		0	MRM	83.3	Positive

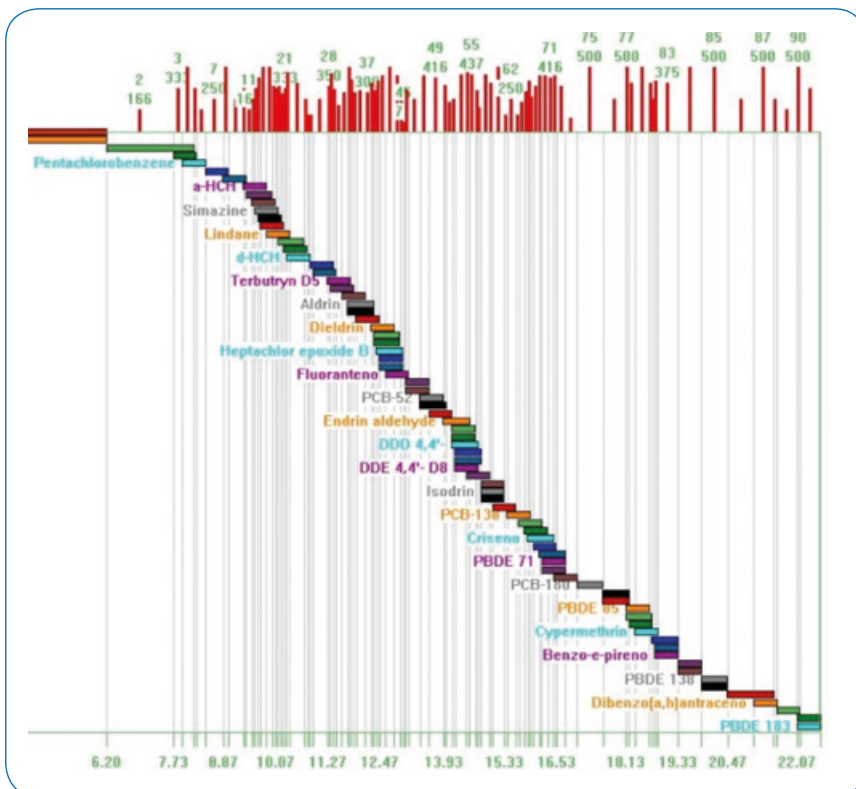|	Precursor	Product	Collision Energy	Q1 Resolution	Q3 Resolution	Scan Time (%)	Qualifier Ion	Qualifier Ratio	Quantifier Ion
1	250.00	215.00	25.00	Standard (2.0	Standard (2.0	50.00%	☐		☑
2	250.00	179.00	25.00	Standard (2.0	Standard (2.0	50.00%	☑	52.80%	☐
3							☐		☐
4							☐		☐

Figure 2: MRM acquisition method

Figure 3: Compound Based Scanning (CBS). Automatic calculation of optimum scan times for each analyzed compound
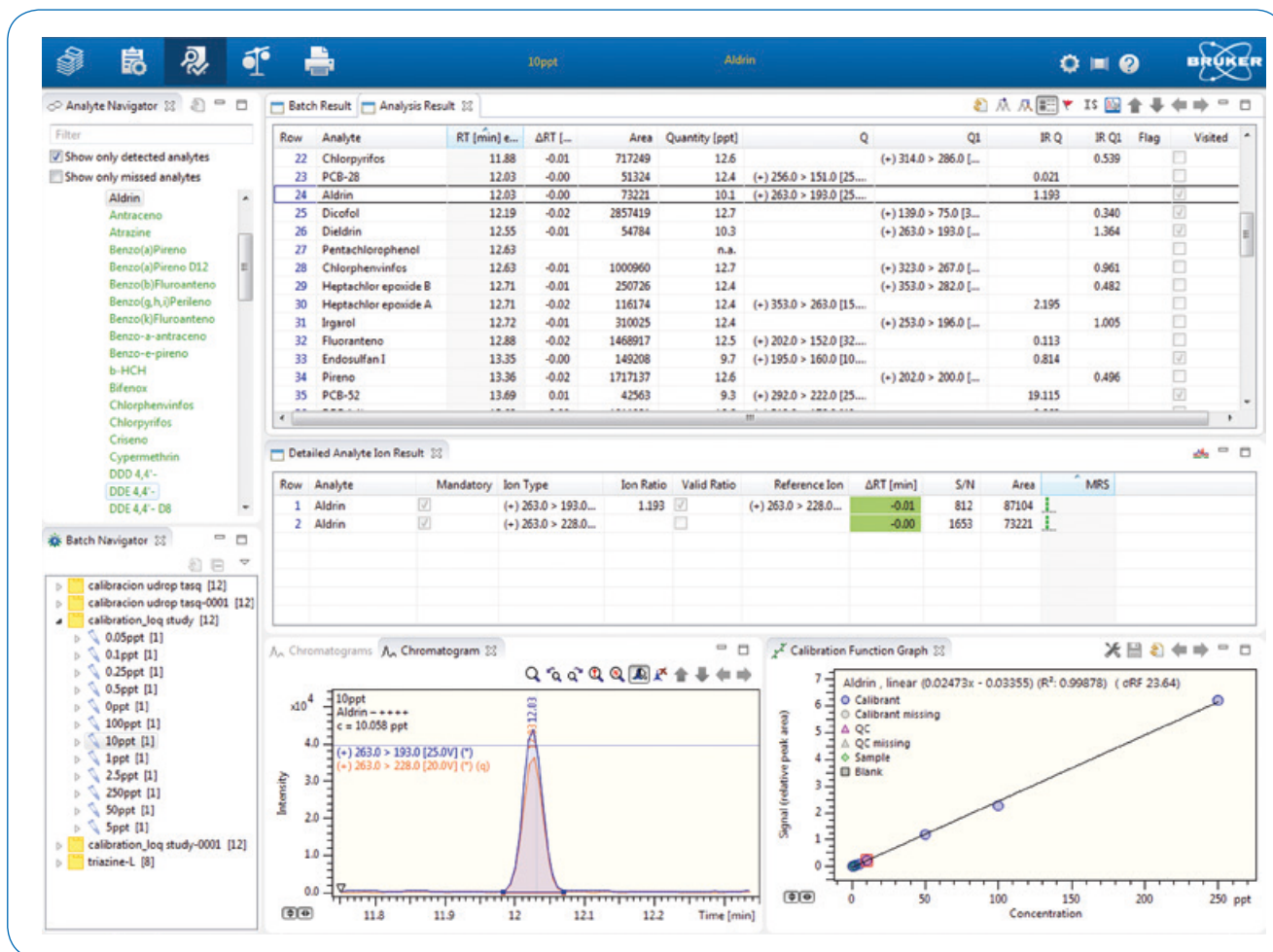
Figure 4: Quick results review using Bruker TASQ 1.4 processing software
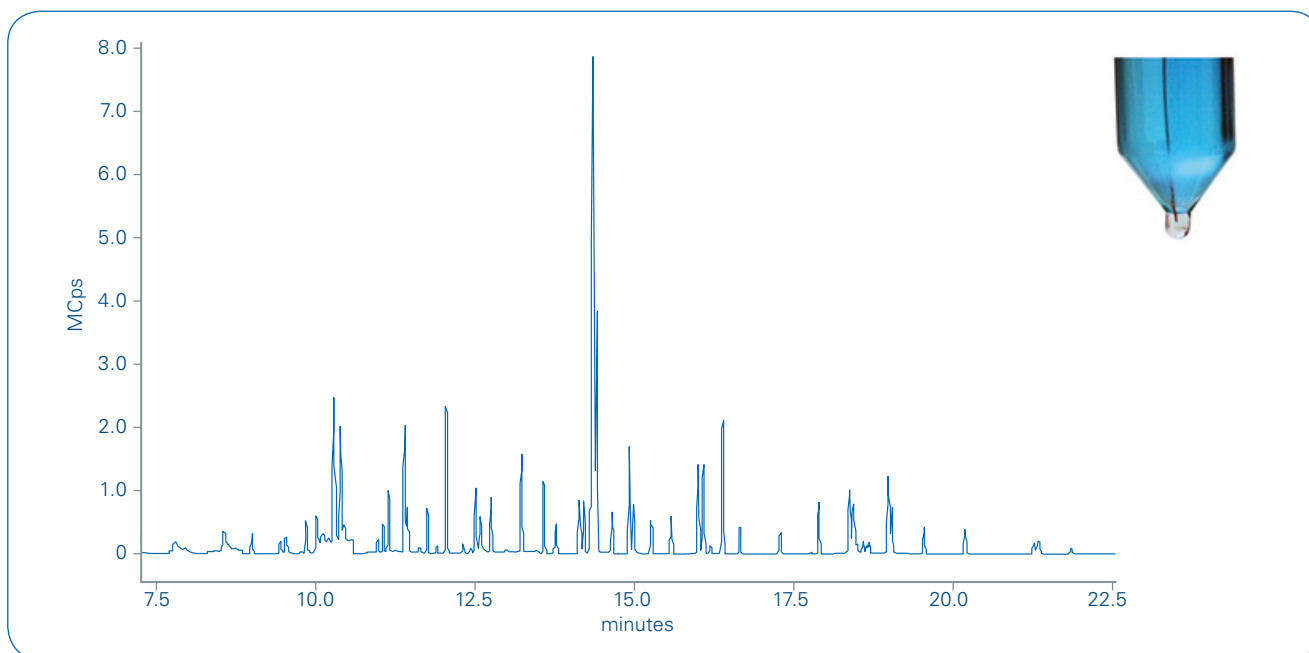

Figure 5: Total ion chromatogram (TIC) of river water sample spiked with 10 ppt of the 62 compounds analyzed. As shown in the photograph in the upper right, the microdroplet (transparent) in the bottom of the tube is clearly distinguishable from the water sample (blue) for easy transfer to the autosampler vial for unattended analysis

Validation study

For method validation, a procedure was followed that ensures the quality of the results, using different water matrices spiked with the compounds to be analyzed and covering a wide range of concentrations, starting with the minimum concentrations for each compound as established in Directive 2013/39/EU.

The parameters set for method validation were as follows:

Matrices and blanks: Selectivity
- ultrapure water [MQ] (Milli-Q® Quality)
- tap water [TAP] (Móstoles, Madrid, Spain, location: 40°20′04.3′′ N; 3°52′55.1′′W)
- river water [RIVER] (Eresma River, Segovia, Spain, location: 40°95′44.02′′ N; -4°12′11.43′′ W)
- sea water [SEA] (Castelldefels, Barcelona, Spain, location: 41° 26′32.28′′ N; 1° 98′57.84′′ W)

Precision and accuracy:
- RSD (≤ 30%) of five repetitions from the low and high working range (specified for each compound in Table 4), independent of matrix. As this method uses procedural standard calibration, only the coefficients of variation were assessed, to be determined on the basis of repeat analysis of calibration standards prepared in each matrix under study.

Linearity:
- Average coefficient of determination ($R^2 > 0.99$) and RSD (< 30%) of the response factor, extracted from four replicates, independent of matrix.

Environmental Quality Standards (EQS) for Priority Substances				
Name of substance	AA-EQS Inland surface waters (ng/L)	AA-EQS Other surface waters (ng/L)	MAC-EQS Inland surface waters (ng/L)	MAC-EQS Inland surface waters (ng/L)
Endosulfan	5	0.5	10	4

Table 3: Example of Endosulfan concentration levels for different surface waters consistent with Directive 2013/39/EU. "Inland surface waters" covers rivers and lakes. "Other surface waters" includes coastal waters. AA = Annual Average, MAC = Maximum Allowable Concentration

Results and Discussion

Linearity

The linearity of the method was assessed using the four water matrices under study. For each matrix, solutions of different concentrations with the 62 analyzed compounds (analytes and deuterated internal standards) were prepared. Each standard sample was then extracted following the sample preparation procedure illustrated in Figure 1. Each concentration level for each matrix was prepared and extracted independently in quadruplicate. Four calibration curve repetitions were therefore performed for each water matrix.

The concentration ranges were set for each analyte, taking two factors into account: calibration curves must have a minimum of five points, and the difference between the high and low working range (coinciding with the low and high points on the curve) must be a minimum of two orders of magnitude. All determinations were performed on extracted samples.

The low working ranges selected were always below the minimum parameters established in Directive 2013/39/EU. An example of the quality parameters for Endosulfan can be found in Table 3. In this example, the low working range was below 0.5 ppt.

Table 4 summarizes the analyzed compounds and the set working ranges. The internal standards (IS) used for each compound were selected on the basis of structural similarity. Example calibration curves are shown in Figure 6.

Lindane
linear 0.05749x - 0.00895
R^2: 0.99913
σRF 17.75

Aldrin
linear 0.02293x - 0.00462
R^2: 0.99858
σRF 19.32

Endosulfan I
linear 0.04425x + 0.02019
R^2: 0.99498
σRF 12.28

Heptachlor
linear 0.2197x - 0.00814
R^2: 0.99931
σRF 11.13

PCB-153
linear 0.2114x - 0.1340
R^2: 0.99982
σRF 10.62

Benzo(a)pyrene
linear 0.1980x + 0.01850
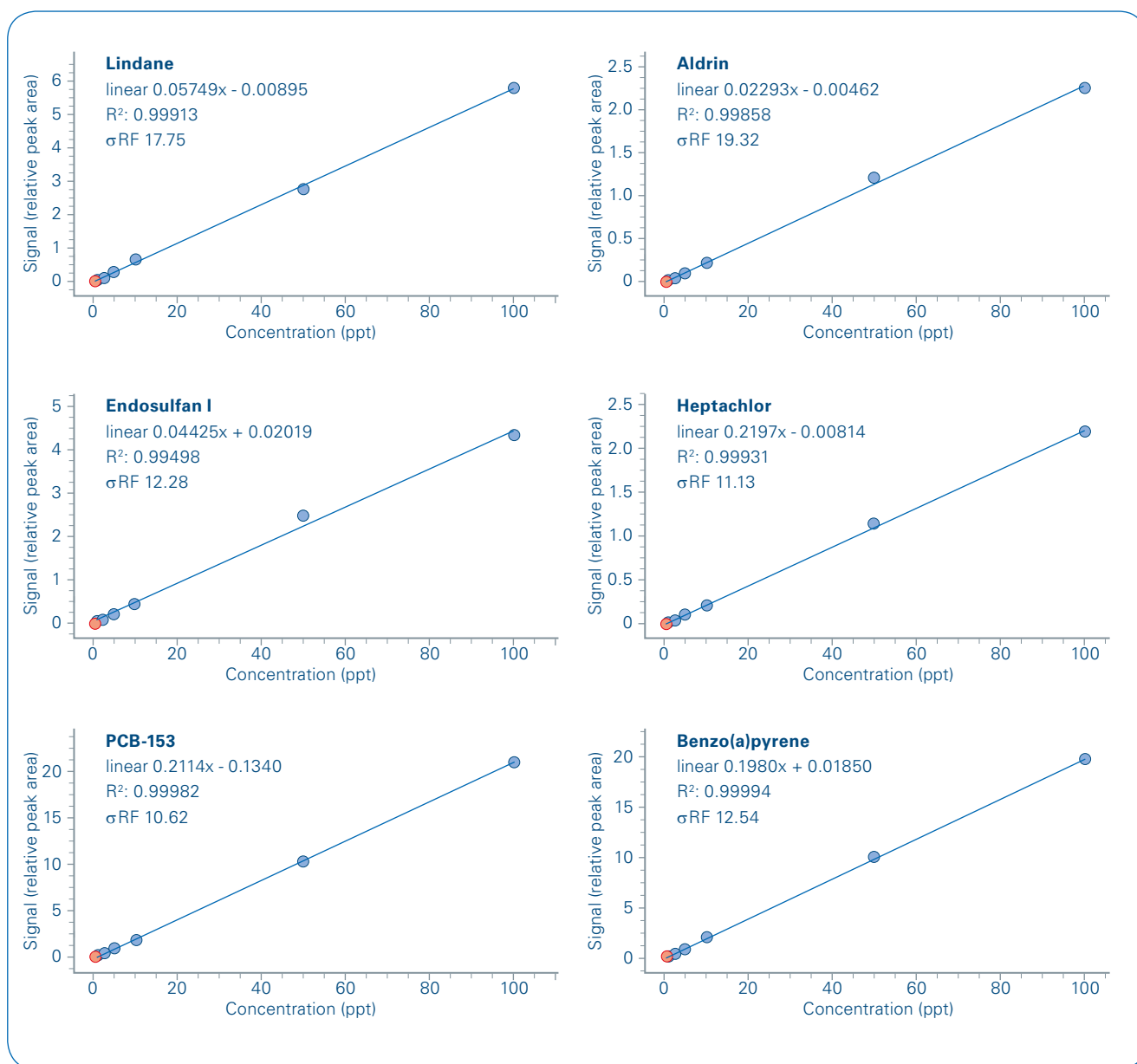R^2: 0.99994
σRF 12.54

Figure 6: Examples of calibration curves for analyzed compounds

	Compounds	RT (min)	Low Working Level (ng/L)	High Working Level (ng/L)	Internal Standard (IS)	Calibration Levels (ng/L)
1	Pentachlorobenzene	8.18	0.50	100	DDE-4,4'- d8	0.5, 1, 5, 10, 50 and 100
2	Trifluralin	9.19	1.00	100	DDE-4,4'- d8	1, 5, 10, 50 and 100
3	α-HCH	9.64	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
4	Hexachlorobenzene	9.74	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
5	Simazine	9.93	5.00	500	Terbutryn-d5	5, 10, 50, 100, 250 and 500
6	Atrazine	9.98	2.50	500	Terbutryn-d5	2.5, 5, 10, 50, 100, 250 and 500
7	β-HCH	10.08	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
8	γ-HCH (Lindane)	10.20	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
9	Anthracene	10.60	2.50	250	Benzo-(a)-pyrene- d12	2.5, 5, 10, 50, 100 and 250
10	δ-HCH	10.68	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
11	Alachlor	11.26	1.00	50	DDE-4,4'- d8	1, 5, 10, 50 and 50
12	Heptachlor	11.45	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
13	Terbutryn-d5 (IS)	11.79	-	-	-	10 for all calibration levels
14	Terbutryn	11.83	0.50	50	Terbutryn-d5	0.5, 1, 5, 10 and 50
15	Chlorpyrifos	11.95	1.00	100	DDE-4,4'- d8	1, 5, 10, 50 and 100
16	Aldrin	12.09	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
17	Dicofol	12.25	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
18	Isodrin	12.61	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
19	Chlorfenvinfos	12.71	1.00	250	DDE-4,4'- d8	1, 5, 10, 50, 100 and 250
20	Heptachlor epoxide B	12.77	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
21	Heptachlor epoxide A	12.87	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
22	Cibutryn	12.89	0.25	50	DDE-4,4'- d8	0.25, 0.5, 1, 5, 10 and 50
23	Fluoranthene	12.96	1.00	100	Benzo-(a)-pyrene- d12	1, 5, 10, 50 and 100
24	Endosulfan I	13.42	0.25	50	DDE-4,4'- d8	0.25, 0.5, 1, 5, 10 and 50
25	DDE-4,4'- d8 (IS)	13.73	-	-	-	10 for all calibration levels
26	DDE-4,4'	13.77	0.50	100	DDE-4,4'- d8	0.5, 1, 5, 10, 50 and 100
27	Dieldrin	13.92	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
28	PCB81	13.98	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
29	Endrin	14.30	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
30	PCB77	14.35	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
31	Endosulfan II	14.50	0.25	50	DDE-4,4'- d8	0.25, 0.5, 1, 5, 10 and 50
32	PCB126	14.51	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
33	PCB123	14.54	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
34	PBDE 28	14.55	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
35	DDD- 4,4'	14.55	0.50	100	DDE-4,4'- d8	0.5, 1, 5, 10, 50 and 100
36	DDT- 2,4'	14.58	0.50	100	DDE-4,4'- d8	0.5, 1, 5, 10, 50 and 100
37	PCB118	14.76	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
38	Aclonifen	14.80	1.00	100	DDE-4,4'- d8	1, 2.5, 5, 10, 50 and 100
39	PCB114	14.90	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
40	Quinoxyfen	15.03	0.50	50	DDE-4,4'- d8	0.5, 1, 5, 10 and 50
41	Endosulfan Sulfate	15.18	0.25	50	DDE-4,4'- d8	0.25, 0.5, 1, 5, 10 and 50
42	DDT- 4,4'	15.21	0.50	100	DDE-4,4'- d8	0.5, 1, 5, 10, 50 and 100
43	PCB105	15.43	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
44	PCB167	15.51	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
45	Bifenox	16.37	0.25	50	DDE-4,4'- d8	0.25, 0.5, 1, 5, 10 and 50
46	PCB169	16.55	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
47	PCB156	16.62	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
48	PBDE 47	16.74	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
49	PCB157	16.84	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
50	PCB189	17.05	0.10	50	DDE-4,4'- d8	0.1, 0.5, 1, 5, 10 and 50
51	PBDE 100	17.58	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
52	PBDE 99	17.85	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
53	Benzo(b)fluoranthene	18.58	0.25	50	Benzo-(a)-pyrene- d12	0.25, 0.5, 1, 5, 10 and 50
54	Benzo(k)fluoranthene	18.64	0.25	50	Benzo-(a)-pyrene- d12	0.25, 0.5, 1, 5, 10 and 50
55	Cypermethrin	18.72	0.25	50	Benzo-(a)-pyrene- d12	0.25, 0.5, 1, 5, 10 and 50
56	Benzo(a)pyrene-d12 (IS)	19.15	-	-	-	1 for all calibration levels
57	Benzo(a)pyrene	19.24	0.10	50	Benzo-(a)-pyrene- d12	0.1, 0.25, 0.5, 1, 5, 10 and 50
58	PBDE 154	19.64	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
59	PBDE 153	19.78	0.10	50	DDE-4,4'- d8	0.1, 0.25, 0.5, 1, 5, 10 and 50
60	Indene(1,2,3-c,d)pyrene	21.53	0.50	50	Benzo-(a)-pyrene- d12	0.5, 1, 5, 10 and 50
61	Dibenzo(a,h)anthracene	21.56	0.50	50	Benzo-(a)-pyrene- d12	0.5, 1, 5, 10 and 50
62	Benzo(g,h,i)perylene	22.13	0.50	50	Benzo-(a)-pyrene- d12	0.5, 1, 5, 10 and 50

Table 4: Analyzed compounds and set working ranges for calibration using the Bruker µDROP method. Deuterated internal standards are shown in red

Figure 7 shows the results of the linearity study of the calibration curves for each of the analyzed compounds in each of the water samples. As shown, the previously set quality criteria are satisfied in all cases: $R^2 \geq 0.99$, with a variation (RSD %) of the curve's response factor $\leq 30\%$ (σRF) for the entire concentration range under study.

The linearity study data reveals several relevant analytics. The first is that 90% of the obtained coefficients of determination (R^2) are above 0.995, meaning that consideration could be given to making this criterion more restrictive should legislation so require. In line with the response factor variation (RSD %), it is noted that the majority of values are grouped together between 10% and 30%. In this case, the previously set criterion is adjusted to align with the analytical reality.

Matrix Effect

Another significant item of note is that with the Bruker µDROP methodology, matrix effects are irrelevant for all matrices under study, facilitating routine application for very different water samples.

To assess any matrix effects, the slope of the calibration curve for MQ water was taken as a reference and compared against the slope of the other water sample curves (TAP, RIVER, and SEA), as illustrated in Figure 8. The difference between the calibration curve slopes varies between 7% and 18%, and the variation coefficient between the slope values is 8.5%. With these results, the laboratory could quantify tap, river, or sea water samples using a calibration curve prepared with pure water.
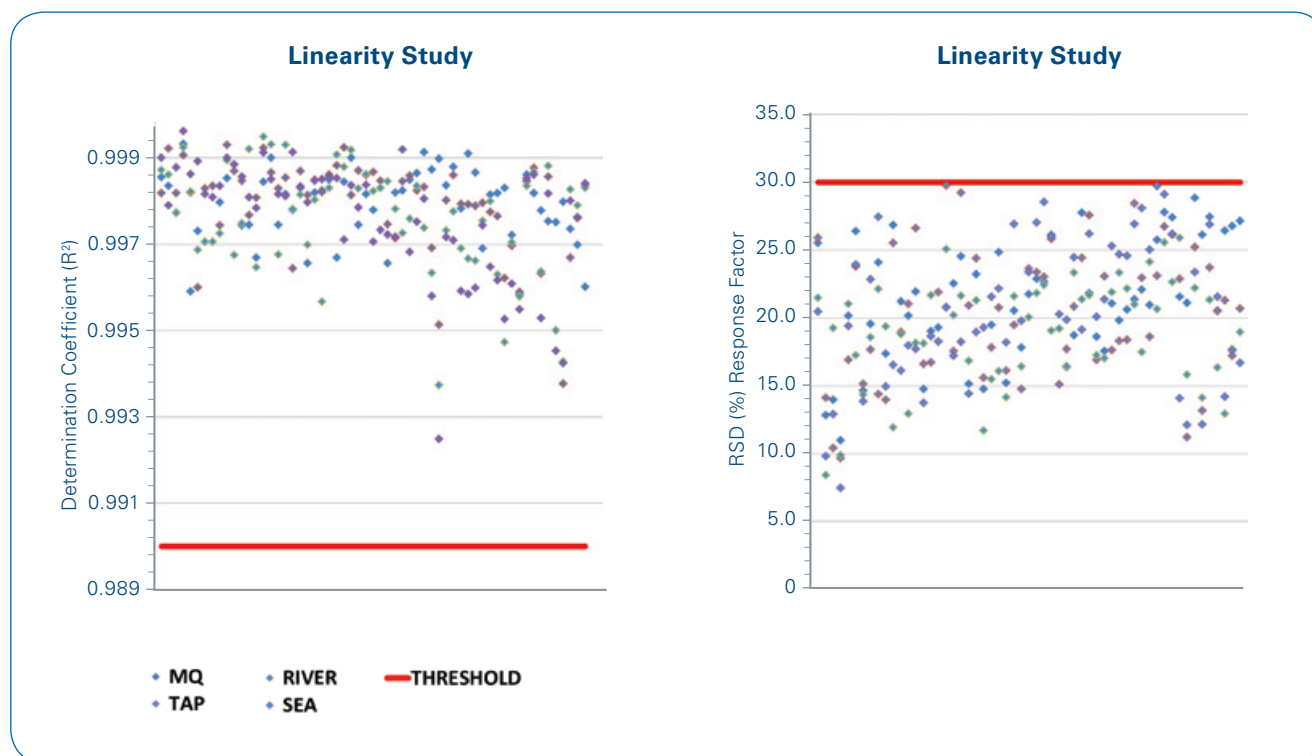

Figure 7: Linearity study: The red lines indicate the limits for the set quality criteria. **Left**: Coefficient of determination (R^2) of the calibration curves of each compound for each of the water matrices under study. **Right**: Relative Standard Deviation (RSD%) of the response factor for the calibration curves of each compound for each of the water matrices under study
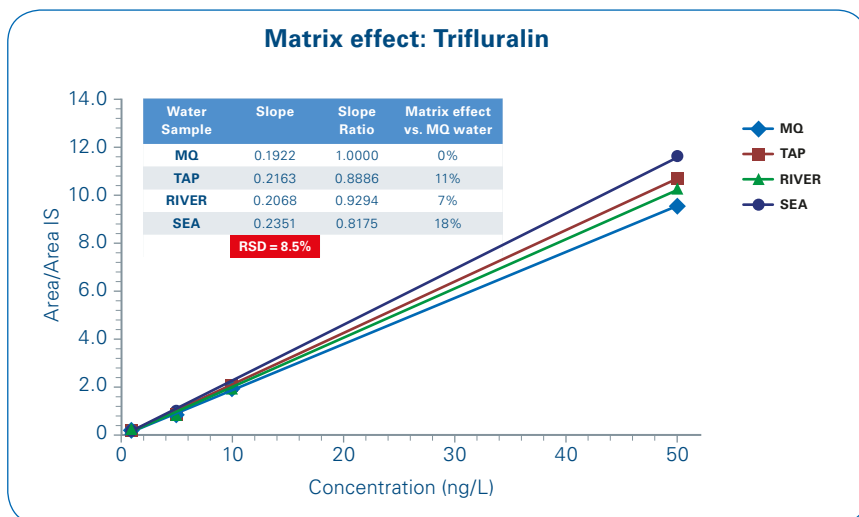
Figure 8: Matrix Effect Assessment: Trifluralin calibration curves in different water matrices
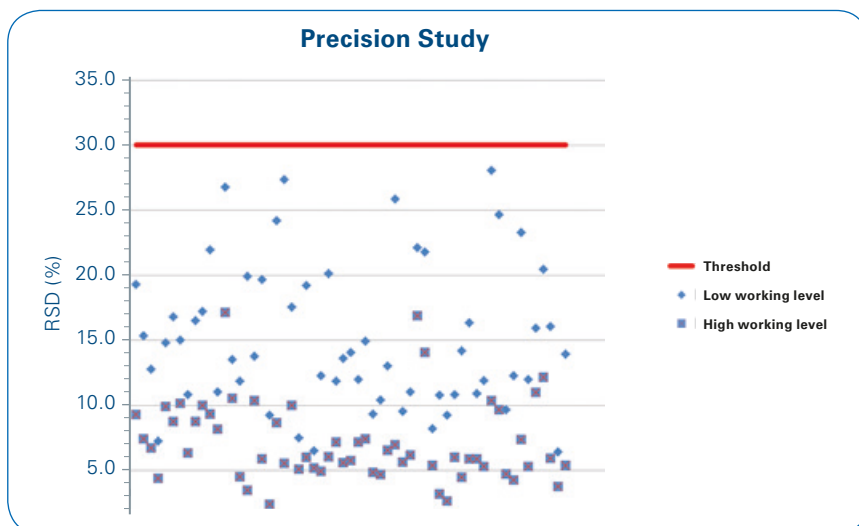

Figure 9: Precision study: RSD (%) of four repetitions at low and high working levels for each of the water samples under study

Precision and Accuracy

As previously established, precision was assessed by determining the dispersal (RSD %) of five repetitions of each of the water samples under study, taken by four different operators across five different days (inter-day precision). Precision was determined for both the low and high working level for each sample. All determinations were performed on extracted samples. The results are shown in Figure 9.

We can observe from the set of results summarized in Figure 9 that no RSD (%) average value exceeds the 30% set as the acceptance criterion. As might be expected, low working ranges offer less precision than high ranges. Most RSD data is within the 5% - 20% range.

In reference to the accuracy, as previously noted, this method uses calibration by extraction of patterns added to the matrix ("procedural standard calibration"). Rather than determine the recoveries in each matrix, the coefficient of variation of repeated analysis of calibration standards prepared in each matrix under study is again determined.

Sensitivity: Method Reporting Limit (MRL) and Method Detection Limit (MDL)

Conventionally, limits of detection have been estimated using the mean of the signal-to-noise ratio for a chromatographic peak with regard to the background of a blank sample and then applying different statistical calculations.

However, modern GC-triple quadrupole MS/MS systems, operating in MRM mode, generate very low background, with near-zero values for many transitions. Clearly, any statistical calculation with near-zero values generates inaccurate estimates of limits of detection that are distant from the analytical reality. This has created a great deal of confusion in establishing limits of detection, and currently published literature unfortunately contains varied definitions, concepts and nomenclatures [8].

An added difficulty in the field of multi-residue analysis in significantly different matrices is that an entirely blank matrix sample is often not available. Therefore, in this work, method sensitivity was experimentally determined using the following two parameters:

- Method Reporting Limit (**MRL**): defined as the lowest concentration that can be reliably quantified meeting the set precision and accuracy criteria. In essence, in this work, the values corresponding to the first calibration point included in the method validation are set as MRLs; in other words, the low working level for each compound described in Table 4.

- Method Detection Limit (**MDL**): defined as the minimum detected concentration of a compound, taking account of sample preparation and the specific method parameters. For each compound, both the quantification and the confirmation ion must be detected with a S/N > 10.

In order to determine the MDLs, extracted water samples spiked with decreasing analyte concentrations were prepared. A blank sample was injected following each analysis, which underwent the same extraction process.

	Compounds	MRL (ng/L)	MDL (ng/L)		Compounds	MRL (ng/L)	MDL (ng/L)
1	Pentachlorobenzene	0.50	0.20	32	PCB126	0.10	0.05
2	Trifluralin	1.00	0.05	33	PCB123	0.10	0.05
3	α-HCH	0.50	0.10	34	PBDE 28	0.10	0.05
4	Hexachlorobenzene	0.50	0.10	35	DDD- 4,4'	0.50	0.05
5	Simazine	5.00	2.50	36	DDT- 2,4'	0.50	0.05
6	Atrazine	2.50	1.00	37	PCB118	0.10	0.05
7	β-HCH	0.50	0.10	38	Aclonifen	2.50	1.00
8	γ-HCH (Lindane)	0.50	0.10	39	PCB114	0.10	0.05
9	Anthracene	2.50	0.05	40	Quinoxyfen	0.50	0.25
10	δ-HCH	0.50	0.10	41	Endosulfan Sulfate	0.25	0.05
11	Alachlor	1.00	0.10	42	DDT- 4,4'	0.50	0.05
12	Heptachlor	0.10	0.05	43	PCB105	0.10	0.05
13	Terbutryn-d5 (IS)	n/a	n/a	44	PCB167	0.10	0.05
14	Terbutryn	0.50	0.05	45	Bifenox	0.25	0.10
15	Chlorpyrifos	1.00	0.05	46	PCB169	0.10	0.05
16	Aldrin	0.50	0.25	47	PCB156	0.10	0.05
17	Dicofol	0.10	0.02	48	PBDE 47	0.10	0.05
18	Isodrin	0.50	0.20	49	PCB157	0.10	0.05
19	Chlorfenvinfos	1.00	0.05	50	PCB189	0.10	0.05
20	Heptachlor epoxide B	0.10	0.05	51	PBDE 100	0.10	0.05
21	Heptachlor epoxide A	0.10	0.05	52	PBDE 99	0.10	0.05
22	Cibutryn	0.50	0.25	53	Benzo(b)fluoranthene	0.25	0.05
23	Fluoranthene	1.00	0.25	54	Benzo(k)fluoranthene	0.25	0.05
24	Endosulfan I	0.25	0.10	55	Cypermethrin	0.25	0.05
25	DDE-4,4'- d8 (IS)	n/a	n/a	56	Benzo(a)pyrene-d12 (IS)	n/a	n/a
26	DDE-4,4'	0.50	0.10	57	Benzo(a)pyrene	0.10	0.05
27	Dieldrin	0.50	0.10	58	PBDE 154	0.10	0.05
28	PCB81	0.10	0.05	59	PBDE 153	0.10	0.05
29	Endrin	0.50	0.10	60	Indene(1,2,3-c,d)pyrene	0.50	0.10
30	PCB77	0.10	0.05	61	Dibenzo(a,h)anthracene	0.50	0.10
31	Endosulfan II	0.25	0.10	62	Benzo(g,h,i)perylene	0.50	0.10

Table 5: The Method Reporting Limits (MRLs) and Method Detection Limits (MDLs) for all analyzed compounds. Values not applicable (n/a) for internal standards
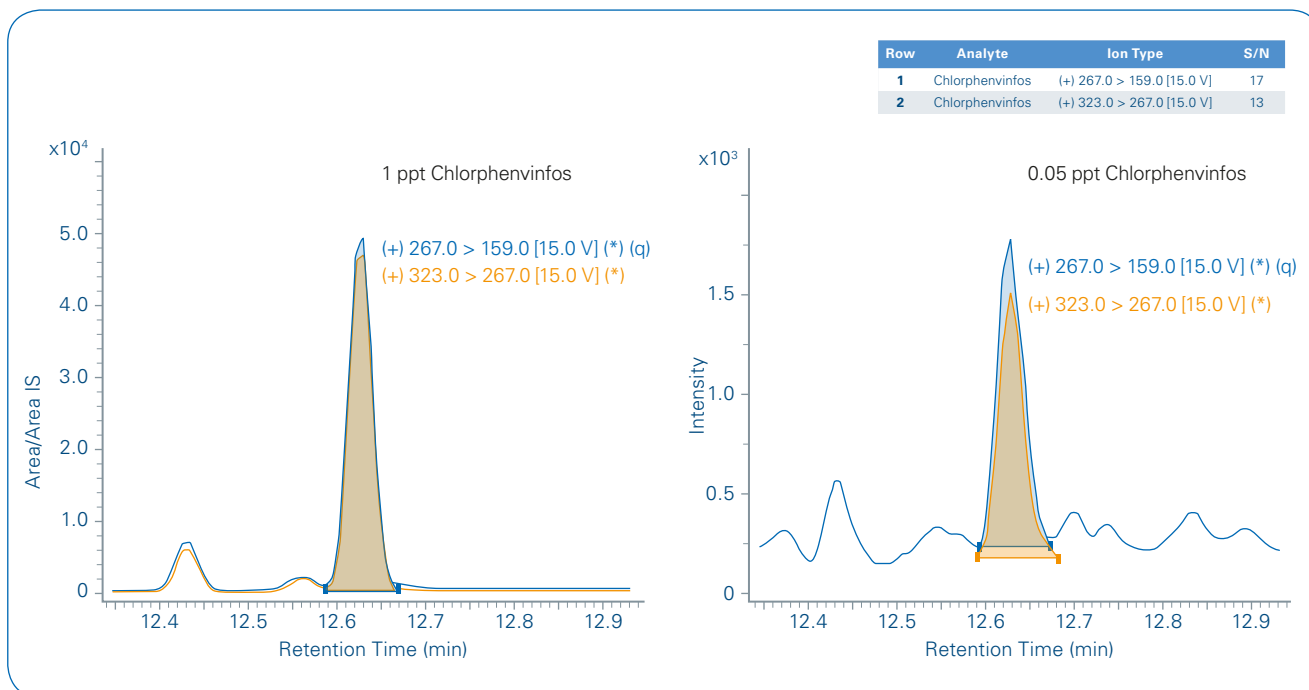
Row	Analyte	Ion Type	S/N
1	Chlorphenvinfos	(+) 267.0 > 159.0 [15.0 V]	17
2	Chlorphenvinfos	(+) 323.0 > 267.0 [15.0 V]	13

Figure 10: Chlorfenvinphos: MRL = 1 ppt (left), MDL=0.05 ppt (right). Blue = quantification ion, Orange = confirmation ion

The MDLs of each compound were set comparing the lowest concentration at which the compound is detectable against the corresponding blank. In this way, realistic MDLs were established. Excessively low estimates prepared using statistical calculations of the signal-to-noise ratio could lead to false positives in routine application.
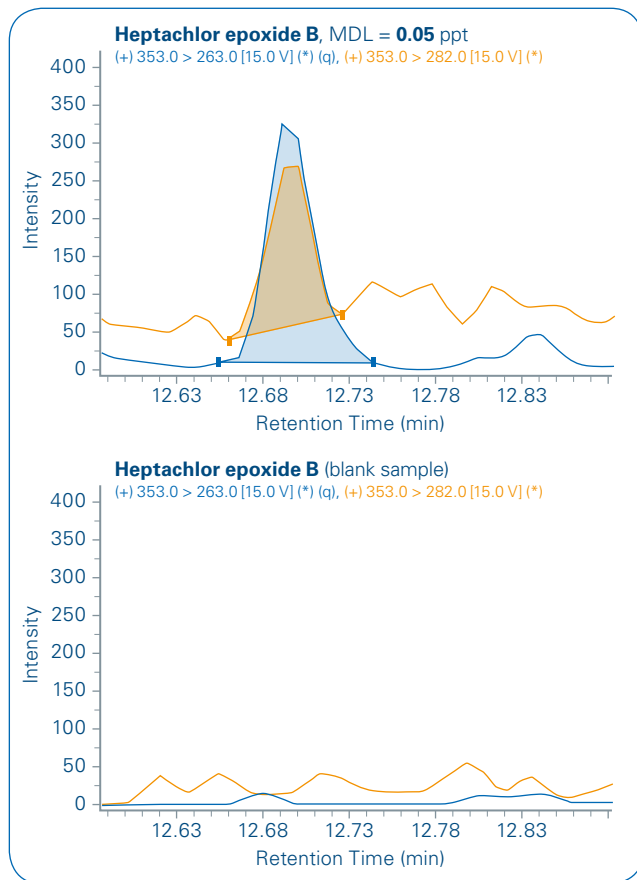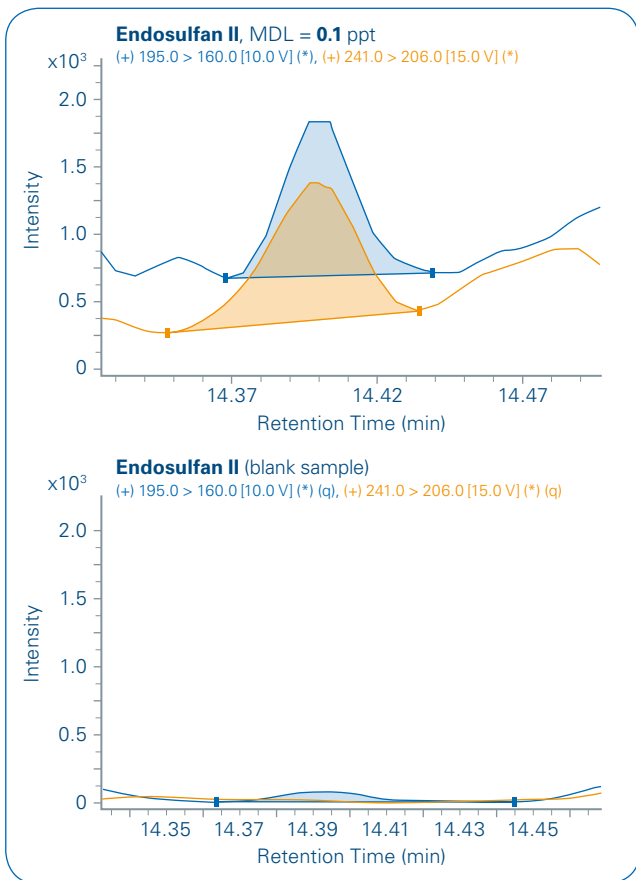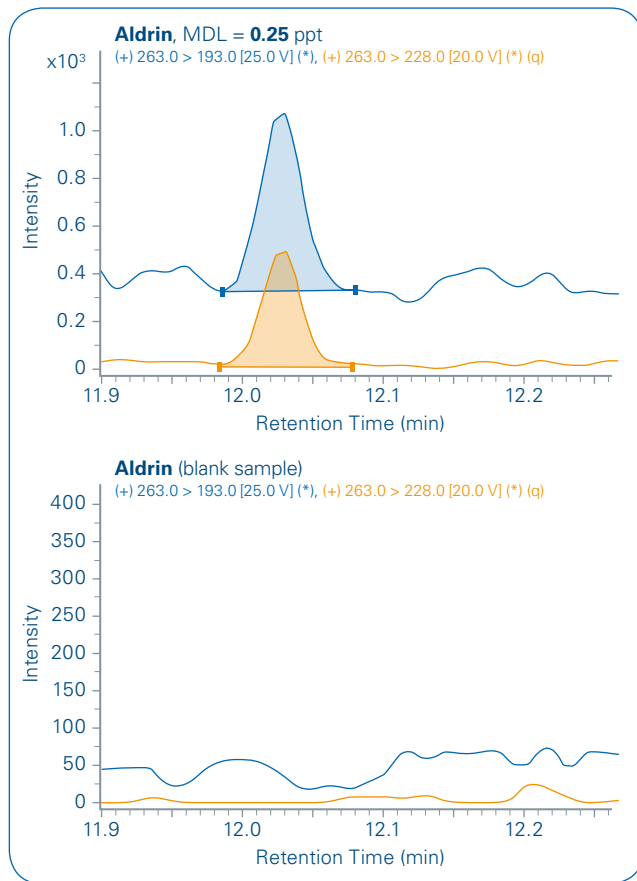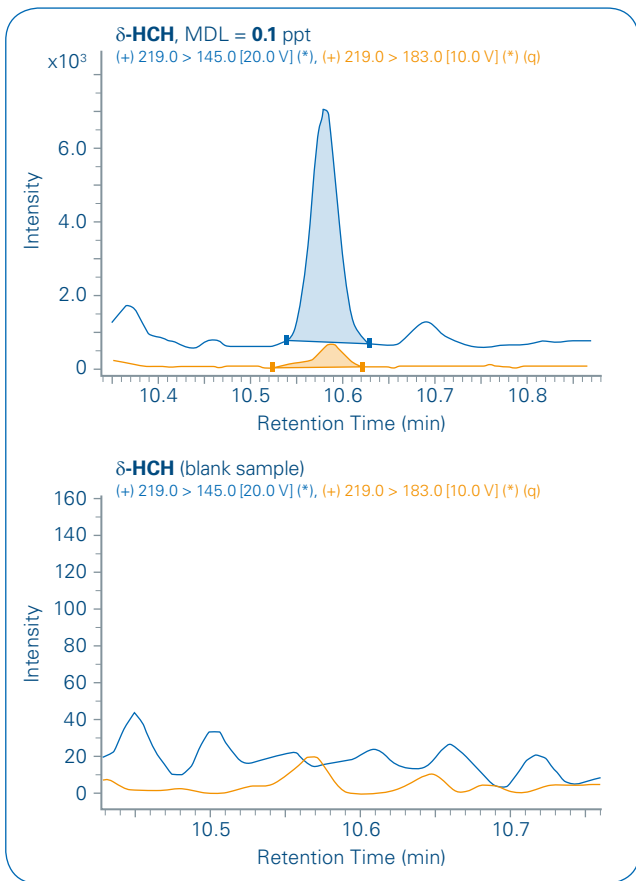
The results of the MRL and MDL values for all analyzed compounds are shown in Table 5.

Figure 10 illustrates the corresponding chromatograms for the set MRL and MDL levels for chlorfenvinphos, showing the quantification and confirmation ions for both levels. Both ions present an S/N > 10 for the MDL level.

Chromatograms for the MDL levels of select compounds in comparison with the corresponding blank samples are shown in Figure 11. Quantification and confirmation ions can be seen in all cases. The compounds are not detected in most of the corresponding blank samples. Traces of Endosulfan II, Benzo(a)pyrene and PBDE 47, however, are detected in the corresponding blank samples, but in concentrations that are well below their set MDL levels.

We may conclude from the sensitivity study that MRLs and MDLs were obtained at low-ppt and sub-ppt levels for all of the analyzed compounds, with realistic and validated limits for routine application of the method.

δ-HCH, MDL = **0.1** ppt
(+) 219.0 > 145.0 [20.0 V] (*), (+) 219.0 > 183.0 [10.0 V] (*) (q)

x10³

Intensity

6.0

4.0

2.0

0

10.4　10.5　10.6　10.7　10.8

Retention Time (min)

δ-HCH (blank sample)
(+) 219.0 > 145.0 [20.0 V] (*), (+) 219.0 > 183.0 [10.0 V] (*) (q)

Intensity

160
140
120
100
80
60
40
20
0

10.5　10.6　10.7

Retention Time (min)

Aldrin, MDL = **0.25** ppt
(+) 263.0 > 193.0 [25.0 V] (*), (+) 263.0 > 228.0 [20.0 V] (*) (q)

x10³

Intensity

1.0
0.8
0.6
0.4
0.2
0

11.9　12.0　12.1　12.2

Retention Time (min)

Aldrin (blank sample)
(+) 263.0 > 193.0 [25.0 V] (*), (+) 263.0 > 228.0 [20.0 V] (*) (q)

Intensity

400
350
300
250
200
150
100
50
0

11.9　12.0　12.1　12.2

Retention Time (min)

Endosulfan II, MDL = **0.1** ppt
(+) 195.0 > 160.0 [10.0 V] (*), (+) 241.0 > 206.0 [15.0 V] (*)

x10³

Intensity

2.0

1.5

1.0

0.5

0

14.37　14.42　14.47

Retention Time (min)

Endosulfan II (blank sample)
(+) 195.0 > 160.0 [10.0 V] (*) (q), (+) 241.0 > 206.0 [15.0 V] (*) (q)

x10³

Intensity

2.0

1.5

1.0

0.5

0

14.35　14.37　14.39　14.41　14.43　14.45

Retention Time (min)

Heptachlor epoxide B, MDL = **0.05** ppt
(+) 353.0 > 263.0 [15.0 V] (*) (q), (+) 353.0 > 282.0 [15.0 V] (*)

Intensity

400
350
300
250
200
150
100
50
0

12.63　12.68　12.73　12.78　12.83

Retention Time (min)

Heptachlor epoxide B (blank sample)
(+) 353.0 > 263.0 [15.0 V] (*) (q), (+) 353.0 > 282.0 [15.0 V] (*)

Intensity

400
350
300
250
200
150
100
50
0

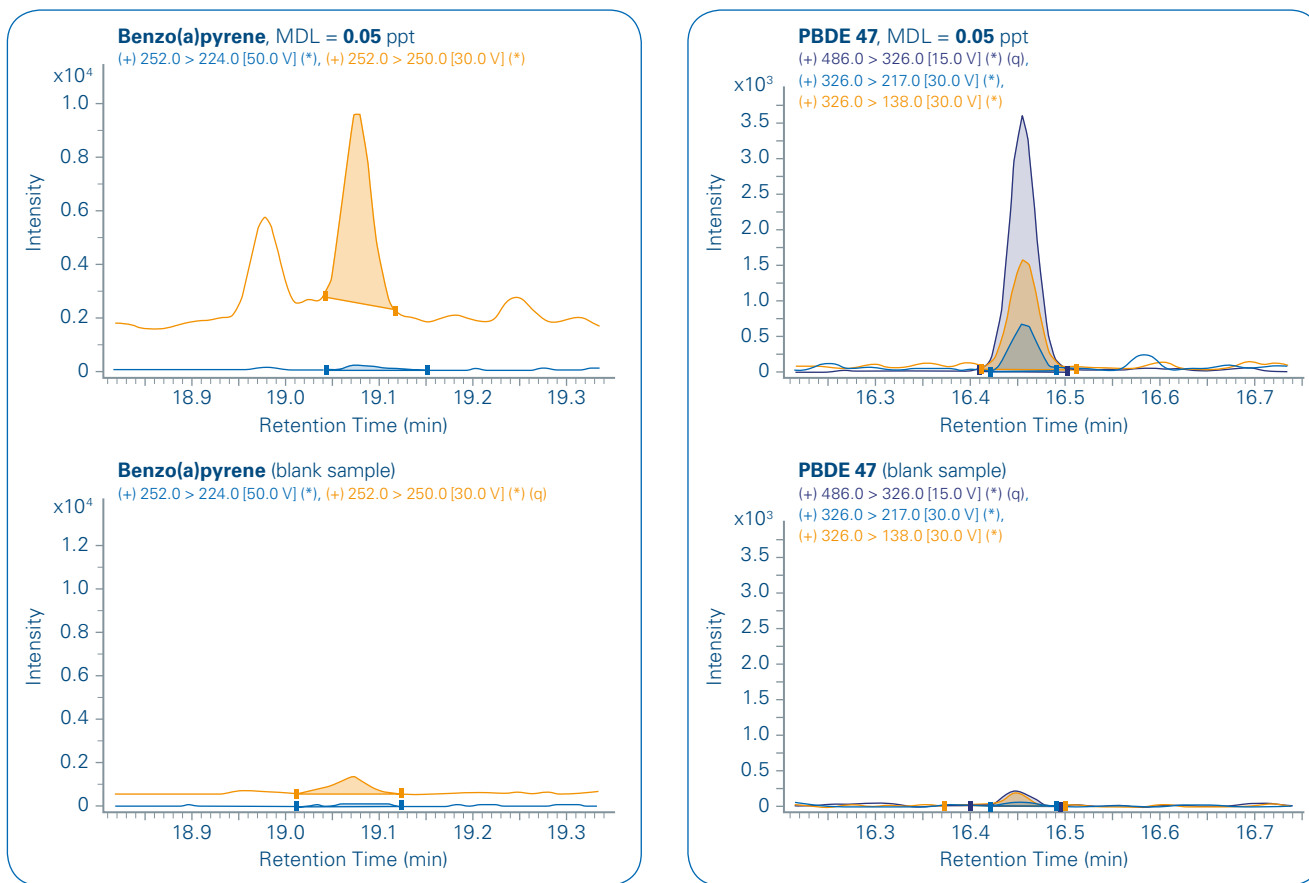12.63　12.68　12.73　12.78　12.83

Retention Time (min)

Figure 11: Sensitivity study. Chromatograms of different compounds at the method detection limit (MDL) set for each compound and comparison with corresponding blank samples. Quantification (q) and confirmation ions can be seen for all compounds

Conclusion

The Bruker µDROP method is a rapid and reliable solution for quality control of surface water and water for human consumption and is ideal for routine application and high productivity in environmental laboratories. It enables ultra-sensitive determination of semi-volatile organic compounds at low-ppt and sub-ppt levels using a reduced sample volume (35 mL). Based on the latest miniaturized microextraction techniques, the Bruker µDROP methodology offers low per-sample cost and minimizes environmental impact in the generation and management of laboratory waste. Using Bruker's powerful EVOQ GC-TQ MS/MS system, the method was validated using matrices and extractions with different operators, obtaining excellent linearity, precision and accuracy values for compliance with the analytical criteria established in the current European directives on water policy.

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Bruker Daltonik GmbH

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com