Sub µg/L Level Analysis of Chlorinated Pesticide and Herbicide Analysis in Water by GC/µECD using Agilent J&W DB-35ms Ultra Inert and DB-XLB columns

Application Note

Environmental

Authors

Doris Smith and Ken Lynam
Agilent Technologies, Inc.
2850 Centerville Rd
Wilmington, DE 19808

Abstract

Chlorinated pesticides and herbicides in water samples are successfully extracted with Agilent SPEC C18AR liquid-solid extraction (LSE) disks. A dual column GC/µECD approach was used employing Agilent J&W DB-35ms Ultra Inert (UI) primary analysis and DB-XLB confirmatory analysis columns. This approach provided consistent and sensitive analysis for the chlorinated compounds at and below established maximum contaminant level concentrations. The method was calibrated over a range of 1 to 100 ng/mL, which corresponds to the expected analyte extraction concentration levels. A water sample fortified at a level of 0.01 µg/L and a tap water sample were extracted and analyzed to demonstrate the effectiveness of this application.

Introduction

Pesticides and herbicides are commonly used in agricultural and residential environments. Pesticide residues are found in many environmental ground and surface waters. These residues enter the water supplies through runoff from pesticide applications and leaching through the soil into groundwater. Human exposure through contaminated drinking water is of concern as pesticides have been linked to serious health and environmental effects. Potential health effects from long term exposure include liver problems and an increased risk of cancer, while recent studies have raised concerns over endocrine disruption [1,2]. The European Union (EU) and United States Environmental Protection Agency (EPA) have established regulations for maximum pesticide levels in drinking water [2,3,4].

Agilent Technologies

Column and liner inertness are critical to achieving consistently reliable analytical results, especially for challenging pesticides such as endrin and DDT, which are particularly susceptible to interaction with active sites in the inlet or on the column [5,6]. Minimizing flow path activity is essential to accurate detection at the trace levels required by the current regulations. This application uses both an Agilent Ultra Inert column and liner to help insure an inert sample flow path.

Quantitative determination of the chlorinated pesticides was achieved by GC/µECD using a dual-column approach. An Agilent J&W DB-35ms Ultra Inert GC column was chosen for primary analysis, while an Agilent J&W DB-XLB column provided confirmatory analysis with a less polar stationary phase than the primary column to help verify the analyte's identity.

The DB-35ms UI offers excellent selectivity for chlorinated pesticides, effectively resolving all thirty-seven of the pesticides and herbicides targeted by the EPA 508.1 method [7]. The EPA 508.1 method recommends pentachloronitrobenzene as an internal standard and 4,4'-dibromobiphenyl as the surrogate standard. Because these two compounds coeleute with analytes of interest, this application was modified by shifting to two surrogate standards commonly used in CLP pesticides analysis, tetra-chloro-m-xylene (TCMX) and decachlorobiphenyl, which are well resolved from the pesticides.

Calibration curve standard preparation can be time consuming and resource intensive. Manual sample preparation can also be prone to errors, resulting in poor reproducibility and precision. The Agilent 7696A Sample Prep WorkBench allows automation of many sample preparation tasks, while significantly reducing solvent use and analysis time. The Agilent 7696A WorkBench has demonstrated high precision and reproducibility, while decreasing variability errors in several sample preparation applications [8,9,10].

The chlorinated pesticides and herbicides are extracted from water using liquid-solid extraction. Because the targeted analytes can be present at trace levels, a large sample volume is needed to extract detectable levels of the pesticides. Current method procedures use a 1 L sample size, which can be time consuming to extract using typical cartridge extractions. Agilent SPEC C18AR 47 mm LSE disks allow faster sample extraction while effectively retaining targeted analytes.

Experimental

An Agilent 7890A Series GC equipped with dual µECD detection and an Agilent 7683B autosampler was used for this study. An inert tee split the effluent 1:1 to the primary and confirmation columns. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies and Table 3 lists the sample preparation supplies.

Table 1. Chromatographic Conditions

Column 1	Agilent DB-35ms UI 30 m × 0.32 mm, 0.25 µm (p/n 123-3832UI)
Column 2	Agilent DB-XLB 30 m × 0.32 mm, 0.5 µm (p/n 123-1236)
GC/µECD	Agilent 7890 Series GC
Sampler	Agilent 7683 automatic liquid sampler, 5.0 µL tapered syringe (p/n 5181-1273)
CFT device	Inert tee (p/n G3184-60065)
Split ratio	1:1
Retention gap	5 m × 0.32 mm id deactivated fused silica tubing
Inlet	2 µL splitless; 250 °C,
Purge flow	60 mL/min at 0.5 min
Carrier	Helium, average velocity 35 cm/s at 80 °C
Oven	80 °C (0.5 min), 26 °C/min to 175 °C, 6.5 °C/min to 235 °C, 15 °C/min to 300 °C (6 min)
µECD	340 °C, constant column + makeup (N_2) = 30 mL/min

Table 2. Flow Path Supplies

Vials and caps	MS certified amber crimp top glass vials and caps kit (p/n 5190-2283)
Vial inserts	250 µL glass/polymer feet (p/n 5181-8872)
Syringe	5 µL tapered (p/n 5181-1273)
Septum	Advanced Green (p/n 5183-4759)
Inlet liner	Ultra Inert single tapered liner (p/n 5190-2292)
Ferrules	0.5 mm id short; 85/15 Vespel/graphite (p/n 5062-3514)
CFT fittings	Internal nut (p/n G2855-20530)
CFT ferrules	SilTite ferrules, 0.32 mm id (p/n 5188-5362)
20x magnifier	20× magnifier loop (p/n 430-1020)

Table 3. Sample Prep Supplies

SPEC disks	Agilent SPEC C18AR 47 mm (p/n A74819)
SPEC manifold system	SPEC 6-position manifold (p/n A712) SPEC disk holders (p/n A713) SPEC 1 L flasks (p/n A714)

Reagents and chemicals

All reagents and solvents were ACS or Ultra Resi grade. Ethyl acetate (EtOAc), methanol (MeOH), and methylene chloride ($MeCl_2$) from JT Baker was purchased through VWR International (West Chester, PA). Hydrochloric acid (HCl) and sodium sulfite (Na_2SO_3) were purchased from Sigma-Aldrich (St. Louis, MO). The EPA 508.1 analyte and surrogate standards were purchased from Ultra Scientific (North Kingstown, RI, USA).

Solutions and standards

An aqueous sodium sulfite solution was prepared at a 50 mg/mL concentration. This solution was added to the sample during collection to reduce any residual chlorine. A 1:1 EtOAc:$MeCl_2$ solution was prepared by mixing equal parts of each solvent.

A 6 N HCl solution was prepared by adding 25 mL hydrochloric acid dropwise to a 50 mL volumetric flask containing approximately 22 mL water in a cooling bath. The solution was allowed to reach room temperature, then diluted to volume with water and mixed thoroughly.

The analyte primary dilution standard was prepared by diluting the commercially prepared pesticide stock solutions with ethyl acetate to yield the analytes at a concentration of 1 µg/mL. This solution was used to fortify a reagent water sample for method analysis. A surrogate standard was prepared at concentrations of 1 µg/mL in ethyl acetate and added to water samples prior to extraction.

The Agilent 7696A Sample Prep WorkBench was used to prepare the calibration standards in ethyl acetate from the neat analyte and surrogate standards over a concentration range of 1 to 100 ng/mL.

Sample preparation

A 1-L water sample was extracted using Agilent SPEC C18AR 47 mm solid-liquid extraction disks, and the extract dried and concentrated prior to GC analysis. Figure 1 illustrates the LSE sample extraction procedure.

A 1-L aliquot of water was collected and 1 mL of 50 mg/mL aqueous Na_2SO_3 was added to convert any residual free chlorine. The pH of the sample was adjusted to pH $\leq$ 2 with 6 N HCl. A quality control sample was spiked with an appropriate amount of spiking solutions to yield a QC sample with an analyte concentration of 0.01 µg/L.

After assembling the vacuum manifold system, the SPEC disk was placed wrinkle side up on the filter. A 5-mL aliquot of 1:1 EtOAc:$MeCl_2$ was added and allowed to soak the disk for 1 minute, then drawn through slowly under vacuum. Next, 5 mL of MeOH was added to the disk and again drawn through slowly, leaving a layer on the disk surface, ensuring the disk did not go dry. The disk was then rinsed with 5 mL of reagent water, which was drawn through under vacuum, again leaving a layer on the disk surface.

A 5-mL aliquot of MeOH was added to the 1-L water sample and mixed well. The appropriate amount of surrogate standard spiking solution was added and the sample shaken. The water sample was drawn through the extraction disk at a rate of about 75 to 100 mL/min. The disk was then dried by drawing air through the disk for about 10 minutes.

The filtration glassware was removed and replaced with a flask containing a collection tube, ensuring the tube fit around the drip tip of the fritted base, and the filtration apparatus reassembled. The sample bottle was rinsed with 5 mL of EtOAc which was transferred to the disk using a disposable pipet. The solvent was drawn through very slowly under vacuum. This bottle rinse step was repeated with 5 mL $MeCl_2$. A glass disposable pipet was used to rinse the filtration reservoir with two 3-mL portions of 1:1 EtOAc:$MeCl_2$.

The eluent was passed through a glass drying tube containing 5 to 7 g anhydrous sodium sulfate. The drying tube was rinsed with two 3-mL portions of 1:1 EtOAc:$MeCl_2$. The extract and washings were collected in a concentrator tube and concentrated to approximately 0.8 mL using a Labconco CentriVap centrifugal concentrator (78100 Series). The inside walls of the tube were rinsed two to three times with EtOAc during concentration. The final extract volume was adjusted to 1.0 mL with EtOAc and transferred to autosampler vials for GC analysis.

Sample extraction procedure

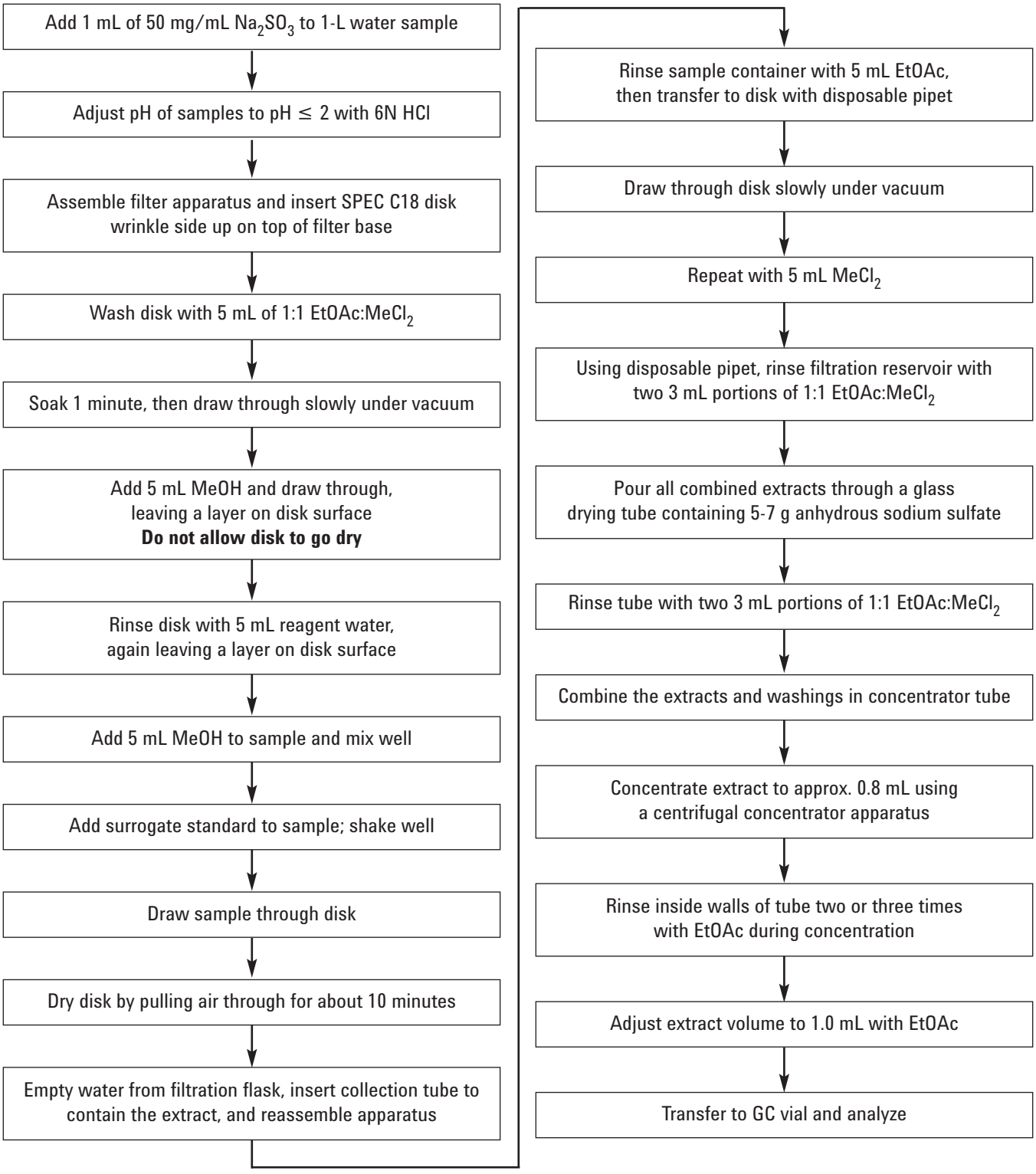

Figure 1. Flow chart for the extraction of chlorinated pesticides in water.

Results and Discussion

The thirty seven targeted chlorinated pesticides and herbicides were resolved on the Agilent DB-35ms UI primary analysis column and the Agilent DB-XLB confirmation column in less than 23 minutes. Figure 2 depicts the dual column GC/μECD chromatograms of a 50 ng/mL standard prepared in ethyl acetate. The enlarged section of the chromatograph in Figure 3 shows the excellent peak response and resolution of a 10 ng/mL EPA 508.1 standard analyzed on the DB-35ms UI column. Figure 4 illustrates the separation and differences in selectivity of the DB-XLB column, demonstrating its benefits as a confirmation column.

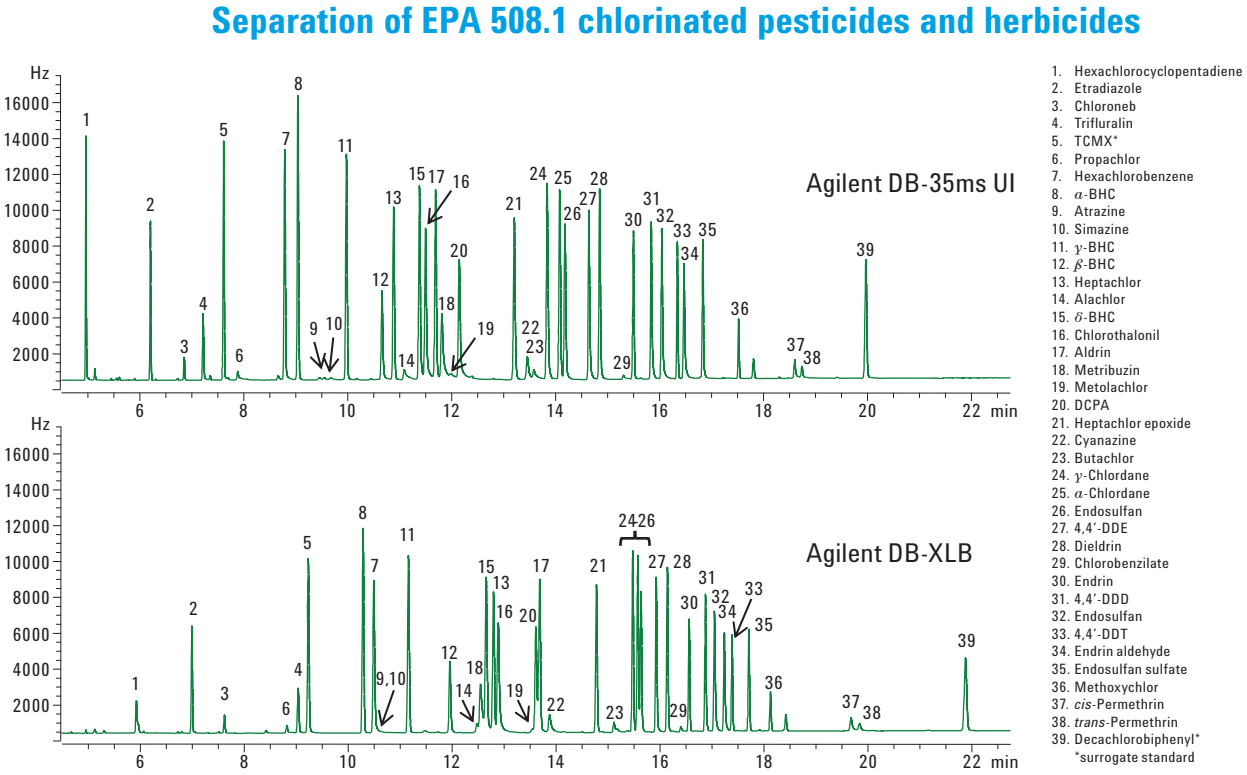

Figure 2. GC/μECD chromatogram of a 50 ng/mL pesticide standard analyzed on an Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 μm column (p/n 123-3832UI) and DB-XLB 30 m × 0.32 mm, 0.5 μm column (p/n 123-1236). This standard was prepared in ethyl acetate using an Agilent 7696A Sample Prep WorkBench. Chromatographic conditions are listed in Table 1.

EPA 508.1 low level pesticides peak shape and resolution with an Agilent DB-35ms UI

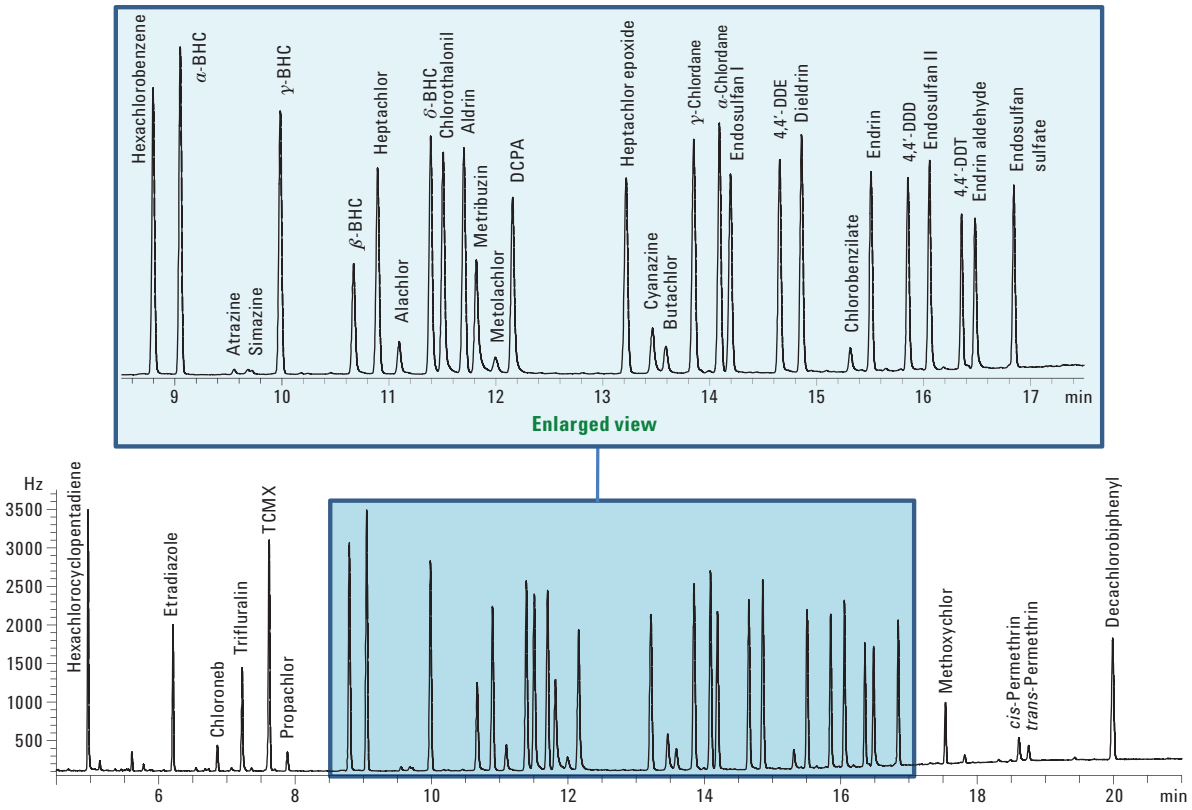

Figure 3. Enlarged section of the GC/μECD chromatogram of a 10 ng/mL chlorinated pesticide standard analyzed on an Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 μm column. The chromatographic conditions are listed in Table 1.

EPA 508.1 low level pesticides peak shape and resolution with an Agilent DB-XLB

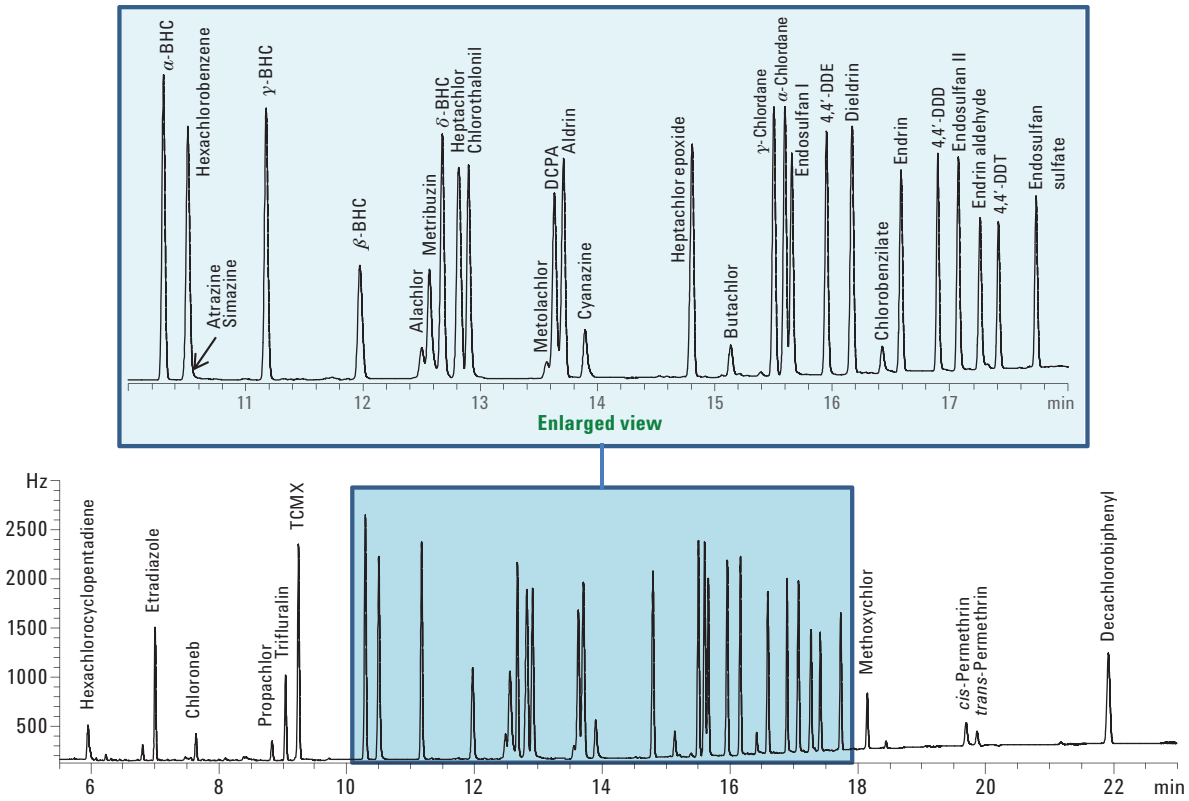

Figure 4. Enlarged section of the GC/µECD chromatogram of a 10 ng/mL chlorinated pesticide standard analyzed on an Agilent J&W DB-XLB 30 m × 0.32 mm, 0.5 µm column. The chromatographic conditions are listed in Table 1.

A seven-point calibration curve was generated to test the linearity of the method. Linearity as defined by the correlation coefficient (R^2) of the calibration curve can be used to evaluate the performance of a gas chromatographic column. The seven-level calibration solutions were prepared by appropriate dilution of commercially prepared standards in ethyl acetate. The Agilent 7696A Sample Prep WorkBench was used to prepare the calibration curve standards at 1, 2.5, 5, 10, 25, 50, and 100 ng/mL.

A nonlinear response can be indicative of breakdown or adsorption of the compound in the inlet or column. The performance of the Agilent DB-35ms UI and Agilent DB-XLB columns yielded correlation coefficient (R^2) values ≥ 0.993 over the calibration range of this study. The individual pesticide analyte values are shown in Table 4.

Table 4. Correlation Coefficients (R^2) for the EPA 508.1 Chlorinated Pesticides Calibration Standards Analyzed by GC/μECD

Linearity results

	R^2 values	
Analyte	**Agilent DB-35ms UI**	**DB-XLB**
Hexachlorocyclopentadiene	0.9996	0.9930
Etradiazole	0.9982	1.0000
Chloroneb	0.9982	0.9981
Trifluralin	0.9976	0.9976
TCMX (ss)	0.9997	0.9997
Propachlor	0.9996	0.9986
Hexachlorobenzene	0.9996	0.9991*
α-BHC	0.9998	1.0000
Atrazine	0.9941	*
Simazine	0.9971	*
γ-BHC	0.9999	0.9998
β-BHC	0.9998	0.9999
Heptachlor	0.9999	0.9998
Alachlor	0.9986	0.9989
δ-BHC	0.9999	0.9996
Chlorothalonil	1.0000	1.0000
Aldrin	0.9998	0.9994
Metribuzin	0.9997	0.9985
Metolachlor	0.9973	0.9987
DCPA	0.9996	0.9998

	R^2 values	
Analyte	**Agilent DB-35ms UI**	**DB-XLB**
Heptachlor epoxide	0.9998	0.9998
Cyanazine	0.9994	0.9998
Butachlor	0.9990	0.9992
γ-Chlordane	0.9998	0.9999
α-Chlordane	0.9998	0.9998
Endosulfan I	0.9998	0.9997
4,4′-DDE	0.9998	0.9998
Dieldrin	0.9998	0.9999
Chlorobenzilate	0.9940	0.9985
Endrin	0.9998	0.9996
4,4′-DDD	1.0000	0.9999
Endosulfan II	0.9999	0.9999
4,4′-DDT	0.9993	0.9996
Endrin aldehyde	1.0000	0.9999
Endosulfan sulfate	0.9997	0.9997
Methoxychlor	0.9993	0.9982
cis-Permethrin	0.9992	0.9992
trans-Permethrin	0.9988	0.9995
Decachlorobiphenyl (ss)	0.9998	0.9997

(ss)-surrogate std *Coelution

The method was able to detect chlorinated pesticides with a high level of sensitivity at trace levels. The European Union Directive sets the content limit of individual pesticides in drinking water at 0.1 μg/L [3]. To reliably achieve this detection level, the method should be capable of a limit of detection (LOD) well below the established threshold. Figure 5 shows an extracted 0.01 μg/L fortified reagent water sample on the Agilent DB-35ms UI and Agilent DB-XLB columns. This sample is fortified at an order of magnitude below the target limit, and is also at or below the maximum contaminant levels (MCLs) established by the EPA for pesticides in drinking water [1].

Sample preparation using Agilent SPEC C18AR liquid-solid extraction disks was effective in retaining and preconcentrating the chlorinated pesticides in the spiked water sample. To determine the trace amounts of pesticides in water at the regulated MCLs, a large sample volume is needed to concentrate the pesticides at a detectable level. The use of the large 47 mm C18 disks enabled extraction of a 1 L water sample at a rate of 75 to 100 mL/min. This allowed samples to be processed in about 10 minutes, reducing sample preparation time and increasing sample throughput.

GC/µECD chromatogram of extracted water samples blank relative to fortified sample

1. Hexachlorocyclopentadiene
2. Etradiazole
3. Chloroneb
4. Trifluralin
5. TCMX*
6. Propachlor
7. Hexachlorobenzene
8. α-BHC
9. Atrazine
10. Simazine
11. γ-BHC
12. β-BHC
13. Heptachlor
14. Alachlor
15. δ-BHC
16. Chlorothalonil
17. Aldrin
18. Metribuzin
19. Metolachlor
20. DCPA
21. Heptachlor epoxide
22. Cyanazine
23. Butachlor
24. γ-Chlordane
25. α-Chlordane
26. Endosulfan
27. 4,4′-DDE
28. Dieldrin
29. Chlorobenzilate
30. Endrin
31. 4,4′-DDD
32. Endosulfan
33. 4,4′-DDT
34. Endrin aldehyde
35. Endosulfan sulfate
36. Methoxychlor
37. *cis*-Permethrin
38. *trans*-Permethrin
39. Decachlorobiphenyl*

*surrogate standard

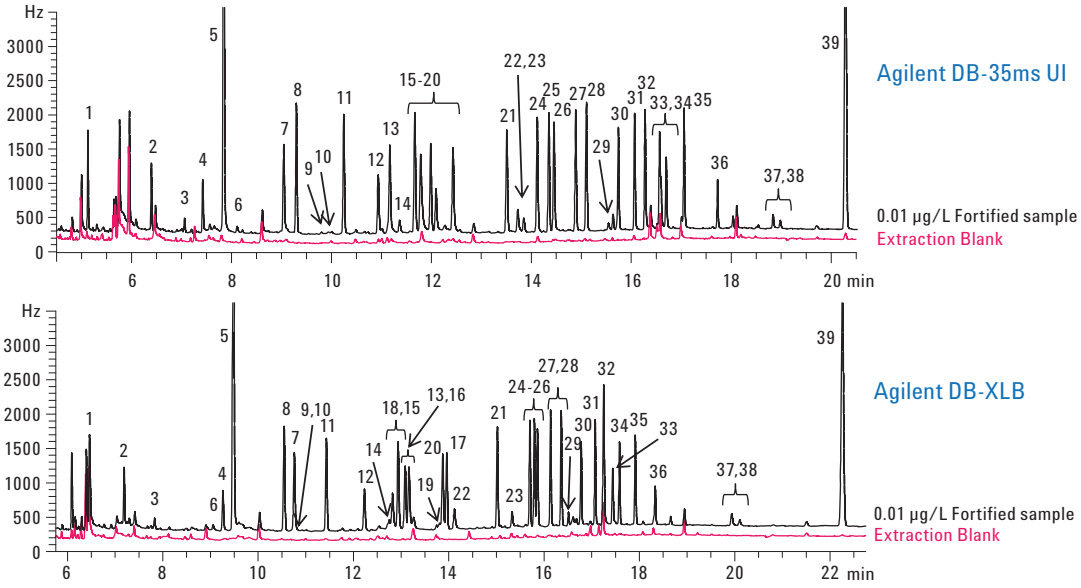

Figure 5. GC/µECD chromatogram for a 0.01 µg/L fortified water sample and extraction blank analyzed using Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 µm column (p/n 123-3832UI) and an Agilent DB-XLB 30 m × 0.32 mm, 0.5 µm column (p/n 123-1236). These samples were prepared and extracted according to the sample preparation procedure detailed in Figure 1. Chromatographic conditions are listed in Table 1.

A drinking water sample was also analyzed for chlorinated pesticides using this method. The tap water sample was collected and prepared according to the sample preparation steps shown in Figure 1 and evaluated under the chromatographic conditions listed in Table 1. The targeted chlorinated compounds were not detected in the tap water sample at the calibrated range of this study. The GC/µECD chromatograms of the sample are shown in Figure 6.

GC/µECD chromatogram of extracted water samples blank relative to drinking water sample

1. Hexachlorocyclopentadiene
2. Etradiazole
3. Chloroneb
4. Trifluralin
5. TCMX*
6. Propachlor
7. Hexachlorobenzene
8. α-BHC
9. Atrazine
10. Simazine
11. γ-BHC
12. β-BHC
13. Heptachlor
14. Alachlor
15. δ-BHC
16. Chlorothalonil
17. Aldrin
18. Metribuzin
19. Metolachlor
20. DCPA
21. Heptachlor epoxide
22. Cyanazine
23. Butachlor
24. γ-Chlordane
25. α-Chlordane
26. Endosulfan
27. 4,4′-DDE
28. Dieldrin
29. Chlorobenzilate
30. Endrin
31. 4,4′-DDD
32. Endosulfan
33. 4,4′-DDT
34. Endrin aldehyde
35. Endosulfan sulfate
36. Methoxychlor
37. *cis*-Permethrin
38. *trans*-Permethrin
39. Decachlorobiphenyl*

*surrogate standard

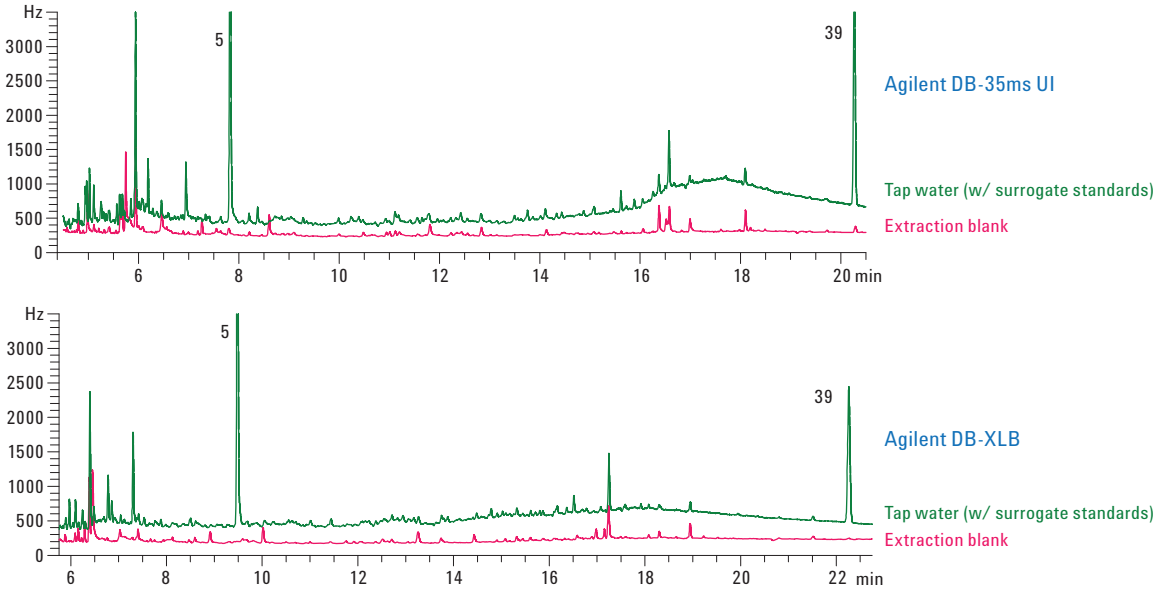

Figure 6. GC/µECD chromatogram for a tap water sample and extraction blank analyzed using Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 µm column (p/n 123-3832UI) and an Agilent DB-XLB 30 m × 0.32 mm, 0.5 µm column (p/n 123-1236). These samples were prepared and extracted according to the sample preparation procedure detailed in Figure 1. Chromatographic conditions are listed in Table 1.

Conclusions

This application note demonstrates an effective analytical method to extract and detect sub-µg/L level chlorinated pesticides and herbicides in water samples. The Agilent J&W DB-35ms UI capillary column adequately resolves all thirty-seven targeted analytes, while providing excellent sensitivity and reliable quantitation at low levels. The separation of the chlorinated pesticides with the Agilent DB-XLB column provides consistent analyte confirmation.

The Agilent SPEC C18AR 47 mm liquid-solid extraction disks successfully extracted and preconcentrated pesticides from water samples, delivering improved trace analyte detection, while reducing sample preparation time. Calibration standards prepared with the Agilent 7696A Sample Prep WorkBench yielded regression coefficients $R^2 \geq 0.993$ for both columns over the range studied.

Pesticide levels were detectable ten fold below the EU and EPA maximum contaminant levels for pesticides in water. A water sample fortified at 0.01 µg/L was successfully prepared and analyzed by this application demonstrating the effectiveness of using Agilent J&W DB-35ms UI and Agilent DB-XLB columns for low level chlorinated pesticide determination. Analysis of a tap water sample did not detect any pesticides at the calibrated levels of this method.

Acknowledgements

The authors thank Dr. Joan Stevens for her help and suggestions with the Agilent LSE procedure.

References

1. Vos JG, Dybing E, Greim HA, Ladefoged O, Lambré C, Tarazona JV, Brandt I, Vethaak AD. (2000) Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit. Rev. Toxicol. 30(1):71-133.

2. EPA. List of Drinking Water Contaminants & MCLs, US Environmental Protection Agency, Washington, DC, Retrieved October 14, 2003 from http://www.epa.gov/safewater/mcl.html#mcls.

3. ECC Council Directive 80/778/ECC. Official Journal of the European Communities, No. L 229, August 30, 1980, p 11.

4. EEC. Drinking Waters Directive, Official Journal N229/11, Directive 80/778/EEC, 1988.

5. Ken Lynam and Doris Smith. Challenging Pesticide Analysis Using an Agilent J&W DB-35ms Ultra Inert GC Column. (2010) Agilent Technologies, Inc. Publication Number 5990-6595EN.

6. Limian Zhao and Allan D. Broske (2010) Evaluation of the Ultra Inert Liner Deactivation for Active Compounds Analysis by GC. Agilent Technologies, Inc. Publication Number 5990-7380EN.

7. US EPA 1995. Method 508.1. Determination Chlorinated Pesticides, Herbicides, and Organohalides by Liquid-Solid Extraction and Electron Capture Gas Chromatography. Revision 2.0.

8. Agilent 7696A Sample Prep WorkBench. (2011) Agilent Technologies, Inc. Publication Number 5990-6908EN.

9. Rebecca Veeneman and Dale Snyder (2010) Improved Data Quality through Automated Sample Preparation. Agilent Technologies, Inc. Publication Number 5990-6974EN.

10. James D. McCurry (2011) Automation of a Complex, Multi-Step Sample Preparation using the Standalone Agilent 7696A WorkBench. Agilent Technologies, Inc. Publication Number 5990-7525EN.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
January 24, 2012
5990-9735EN

Agilent Technologies