



Automated Solid Phase Extraction and Gas Chromatography-Mass Spectrometry Analysis of Tap Water Samples According to EN 16693:2015 for Organochlorine Pesticides

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Key Words

OCPs, EN 16693:2015, Water Framework Directive, Priority Substances, Disk-Based SPE, whole water samples

Introduction

The Water Framework Directive (WFD) is a European Union directive adopted in 2000 by the European Commission which commits European Union member states to protect and restore clean water across Europe by 2015. The framework comprises the development of a list of priority substances and analytical methodologies to control and monitor in a consistent and comparable way the pollution of different water bodies at the Environment Quality Standard (EQS) level. Directive 2013/39/EU¹ introduced in August 2013 amends Directives 2000/60/EC and 2008/105/EC and limits the concentration of 45 priority substances. Among these 45 priority substances, 13 are organochlorine pesticides (OCPs, Table 1) and among them, 12 are also classified as hazardous substances under the WFD, i.e., toxic, persistent, and liable to bioaccumulations. To support the implementation of the WFD, the European Committee for Standardization (CEN) published in 2015 a European standard EN 16693:2015² that describes and specifies a procedure to determine 21 selected OCPs in whole water samples using the solid phase extraction (SPE) coupled with gas chromatography-mass spectrometry (GC-MS).

Organochlorine pesticides are synthetic pesticides that belong to the large group of chlorinated organics composed primarily of carbon (C), chlorine (Cl), and hydrogen (H). These chlorinated hydrocarbons have strong polar covalent bonds in their chemical structures between their chlorine and carbon atoms (327 kJ/mol), thus making them non-biodegradable, inert, and extremely persistent in the environment. The chlorine atoms in their structures also confers them specific chemical properties such as low water solubility and a high affinity for fats. Therefore, OCPs tend to absorb onto particles and accumulate in lipid-rich tissue of animals, including humans and aquatic life. By their nature, OCPs are potentially toxic. Numerous studies have linked OCPs exposures with cancers, endocrine disruptor effects, neurotoxic and reproductive effects.

OCPs have been used for several applications (especially as insecticides) and were widely dispersed into the environment over the past decades. In 1942, during World War II, Dichlorodiphenyltrichloroethane (DDT) was used to control Malaria and Typhus among civilians and troops. Although first synthesized in 1874, the insecticidal action of DDT was discovered in 1939 by the Swiss chemist Paul Hermann Müller who also received a Nobel prize in 1948 for this discovery.

This note demonstrates the performance of automated disk-based solid phase extraction (SPE) for concentration of organochlorine pesticides from whole water, prior to GC/MS analysis, following the requirements of EN16693:2015.

APPLICATION NOTE



Table 1. Substance, Molecular Formula and CAS Numbers of the OCPs Determined by EN 16693:2015

| Substance | Molecular Formula | CAS # | Priority Substance under WFD | Priority Hazardous Substance under WFD |
|-------------------------------|---|------------|------------------------------|--|
| Alachlor | C ₁₄ H ₂₀ ClN ₂ O ₂ | 15972-60-8 | x | |
| Cyclodiene pesticides: | | | | |
| Aldrin | C ₁₂ H ₈ Cl ₆ | 309-00-2 | | |
| Dieldrin | C ₁₂ H ₈ Cl ₆ O | 60-57-1 | | |
| Endrin | C ₁₂ H ₈ Cl ₆ O | 72-20-8 | | |
| Isodrin | C ₁₂ H ₈ Cl ₆ | 465-73-6 | | |
| DDT-total: | | | | |
| op'-DDT | C ₁₄ H ₉ Cl ₅ | 789-02-6 | | |
| pp'-DDT | C ₁₄ H ₉ Cl ₅ | 50-29-3 | | |
| pp'-DDD | C ₁₄ H ₉ Cl ₄ | 72-54-8 | | |
| pp'-DDE | C ₁₄ H ₉ Cl ₄ | 72-55-9 | | |
| Hexachlorobenzene (HCB) | C ₆ Cl ₆ | 118-74-1 | x | x |
| Hexachlorobutadiene (HCBD) | C ₄ Cl ₆ | 87-68-3 | x | x |
| Hexachlorocyclohexane: | | | | |
| alpha-HCH | C ₆ H ₆ Cl ₆ | 319-84-6 | x | x |
| beta-HCH | C ₆ H ₆ Cl ₆ | 319-85-7 | x | x |
| delta-HCH | C ₆ H ₆ Cl ₆ | 319-86-8 | x | x |
| gamma-HCH | C ₆ H ₆ Cl ₆ | 58-89-9 | x | x |
| Pentachlorobenzene | C ₆ HCl ₅ | 608-93-5 | x | x |
| Trichlorobenzene: | | | | |
| 1,2,3-TCB | C ₆ H ₃ Cl ₃ | 87-61-6 | x | x |
| 1,2,4-TCB | C ₆ H ₃ Cl ₃ | 120-82-1 | x | x |
| 1,3,5-TCB | C ₆ H ₃ Cl ₃ | 108-70-3 | x | x |
| Endosulfan: | | | | |
| Endosulfan-I (alpha) | C ₉ H ₆ Cl ₆ O ₃ S | 959-98-8 | x | x |
| Endosulfan-II (beta) | C ₉ H ₆ Cl ₆ O ₃ S | 33213-65-9 | x | x |

Experimental

Chemicals

All solvents used were HPLC grade or higher. Target substances were purchased from Restek and Supelco. Mass-labelled internal standards were purchased from Chiron. Stock standard solutions, 2 µg/mL, were prepared in methanol and stored at 4°C. Calibration solutions and working standards were prepared from stock solutions by dilution with methanol and stored at 4°C.

Chemical Summary

1. Acetone \geq 99.8% HiPerSolv CHROMANORM® for HPLC.
2. Hexane \geq 97% HiPerSolv CHROMANORM® for HPLC.
3. Water AnalaR NORMAPUR® ISO 3696, Grade 3 analytical reagent.

Instrumentation

- Horizon Technology, Inc.
 - SPE-DEX® 5000 Automated Extractor
 - XcelVap® Evaporation/Concentration System
 - SDS-101 Solvent Drying System
 - DryDisk® Membranes
 - Atlantic® DVB SPE Disk
- Thermo Fisher Scientific
 - TRACE™ GC 1300
 - Programmed Temperature Vaporizer (PTV) Inlet
 - ISQ™ LT Single Quadrupole Mass Spectrometer
 - TriPlus RSH™ Autosampler
- Restek
 - Rxi™-5ms Capillary Column, 30 m x 0.25 mm ID, 0.25 μ m film thickness



Shown: SPE-DEX 5000 Extraction System

Sample Preparation

All water samples were transferred into a 1 liter glass-bottle container (laboratory bottle with DIN thread, GL 45). The reversed-phase polymeric sorbents evaluated in this work is divinylbenzene (DVB). Sample extraction was performed during the same sampling day as follows:

1. Transfer 1 liter of sample into a glass-bottle container.
2. Spike the mass-labelled internal standard (500 ng) into sample.
3. Spike the target substances (200 ng) into sample.
4. Incorporate the 47-mm disk holder assembly loaded with an Atlantic® DVB SPE Disk.
5. Extract the sample using the SPE-DEX® 5000 Automated Extraction System and the method shown below in Table 2.
6. Collect and dry the extract using the SDS-101 Solvent Drying System loaded with a DryDisk® Separation Membrane.
7. Concentrate the dried extract using the XcelVap® Concentration System and the method shown in Table 3 to a final volume of 1 mL.
8. Transfer the concentrated extract to a 2 mL glass-GC vials and perform the final analysis using GC-MS.

Table 2. Automated Extraction Method

| Step | Operation | Solvent | Solvent Vol. (mL) | Purge Time (s) | Pump Rate (#) | Sat. Time (s) | Soak Time (s) | Drain Time (s) | |
|------|------------------------|----------------------|-------------------|----------------|---------------|-------------------------------|------------------------|----------------|----------------|
| 1 | Condition SPE Disk | Hexane | 10 | 30 | 2 | 1 | 30 | 30 | |
| 2 | Condition SPE Disk | Acetone | 10 | 30 | 2 | 1 | 30 | 30 | |
| 3 | Condition SPE Disk | Reagent Water | 10 | 30 | 2 | 1 | 30 | 30 | |
| 4 | Condition SPE Disk | Reagent Water | 10 | 30 | 2 | 1 | 30 | 30 | |
| Step | Operation | Sample Flow Rate (#) | | | | Done Loading Sample Delay (s) | | | |
| 5 | Load Sample | 3 | | | | 45 | | | |
| Step | Operation | Solvent | Solvent Vol. (mL) | Purge Time (s) | Pump Rate (#) | N ₂ Blanket | Sat. Time (s) | Soak Time (s) | Drain Time (s) |
| 6 | Wash Sample Container | Reagent Water | 15 | 60 | 6 | Off | 1 | 10 | 15 |
| Step | Operation | Dry Time (s) | | | Pump Rate (#) | | N ₂ Blanket | | |
| 7 | Air Dry Disk Timer | 360 | | | 6 | | Off | | |
| Step | Operation | Solvent | Solvent Vol. (mL) | Purge Time (s) | Pump Rate (#) | N ₂ Blanket | Sat. Time (s) | Soak Time (s) | Elute Time (s) |
| 8 | Elute Sample Container | Acetone | 5 | 60 | 2 | Off | 1 | 30 | 30 |
| 9 | Elute Sample Container | Hexane | 10 | 30 | 2 | Off | 1 | 30 | 30 |
| 10 | Elute Sample Container | Hexane | 10 | 30 | 2 | Off | 1 | 30 | 30 |
| 11 | Elute Sample Container | Hexane | 10 | 30 | 6 | Off | 1 | 30 | 120 |

Table 3. XcelVap Concentrator System Settings

| | |
|-------------------------|--|
| Extract Starting Volume | 45 mL of Acetone:Hexane in a 8:1 ratio |
| Water Bath Temperature | 60 °C |
| Nitrogen Ramp | 14 minutes at 0.14 – 0.97 bar (1 step) |
| Gas source | 5 bar of Nitrogen from gas cylinder |

GC-MS Analysis

The separation of the 21 OCPs was carried out on a Restek Rxi-5ms capillary column (30 m x 0.25 mm ID, 0.25 μm film thickness) using a Thermo Scientific TRACE GC 1300 equipped with a single quadrupole mass spectrometer ISQ LT (Thermo Scientific) and a TriPlus RSH autosampler. The analytical conditions are presented in Table 4.



Shown: XcelVap Evaporation/
Concentration System

Table 4. GC and MS Conditions

| Parameter | Setting | Remark(s) |
|--|------------------|-------------------|
| Autosampler | | |
| Volume injected (μL) | 1 | |
| Flush A | | Hexane |
| Injection | | |
| Type | PTV | |
| Mode | PTV splitless CT | |
| Temp ($^{\circ}\text{C}$) | 280 | |
| Splitless time (min) | 1 | |
| Split flow (ml/min) | 50 | |
| Oven | | |
| Initial temperature ($^{\circ}\text{C}$) | 45 | |
| Initial time (min) | 1 | |
| Final temperature ($^{\circ}\text{C}$) | 300 | |
| Final time (min) | 2 | |
| Rate ($^{\circ}\text{C}/\text{min}$) | 20 | |
| Carrier | | |
| Type | Helium | |
| Mode | Constant flow | |
| Flow (mL/min) | 0.9 | |
| Detection | | |
| Type | MS | |
| Mode | EI | |
| Acquisition | MS full scan | Range: 45-450 amu |
| Electron energy (eV) | 70 | |
| Transfer line temperature ($^{\circ}\text{C}$) | 300 | |
| Source temperature ($^{\circ}\text{C}$) | 280 | |

Results and Discussion

Duplicate tap water samples were spiked with target substances at 200 ng/L, extracted by disk-based SPE, and analyzed by GC-MS. An extra tap water sample was run as blank to show if any existing target OCPs was present. All of the tap water used in this work was collected the same day from the same source. Quantification and method validation were determined with recovery experiments based on ion peak areas of selected ions as specified in the EN 16693:2015 method. Target substance recovery values were calculated using the calibration curve combined with the internal standard method to correct any minor deviations due to sample losses throughout the sample preparation and injection. All water samples were spiked with mass-labelled internal standards at 500 ng/L, extracted by disk-based SPE, and analyzed by GC-MS. All water samples were processed in approximately 10 minutes, thus giving an average sample flow rate of 100 mL/min during the loading step. The total time for the OCPs extraction method (conditioning, loading, washing, air-dry, and elution) on the SPE-DEX 5000 was 38 minutes. A typical chromatogram with peak assignments and retention times is depicted Figure 1 / Table 5. The recovery study and overall results are shown in Table 6. The blank measurement confirms non-detected (ND) target substances in the Belgian tap water used in the frame of this study. The precision was excellent overall and all results were above 70% for the four internal standards. The recovery of each target substance complies with the method requirement.

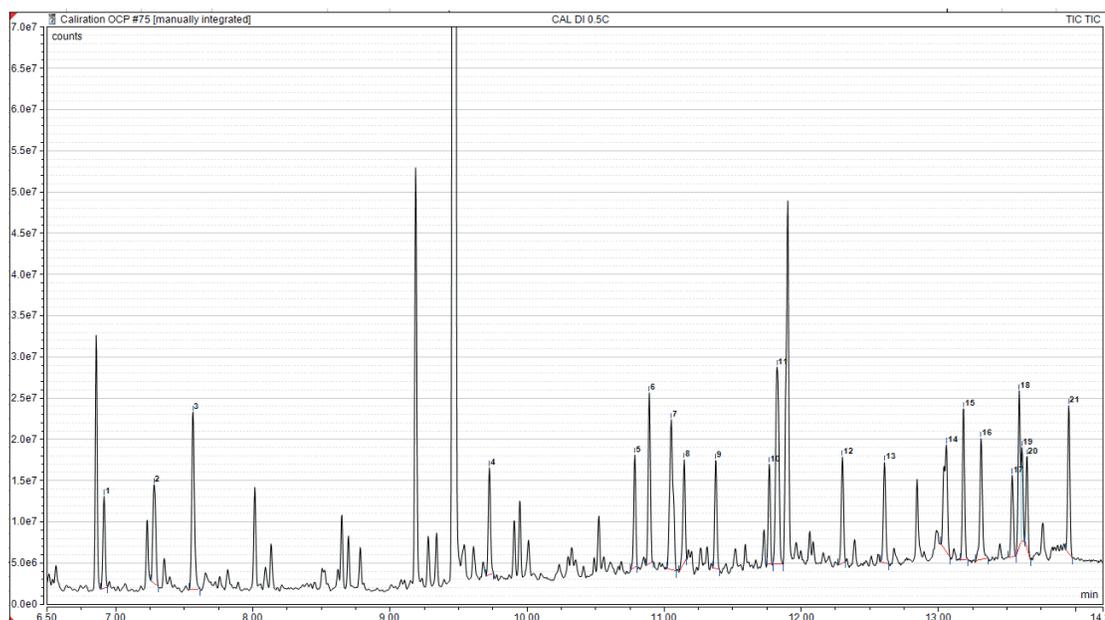


Figure 1. Gas Chromatogram of 21 OCPs.

Table 5. Retention Times

| Peak number | Rt (min) | Identification |
|-------------|----------|--|
| 1 | 6.9 | 1,3,5-TCB |
| 2 | 7.27 | 1,2,4-TCB |
| 3 | 7.56 | 1,2,3-TCB + Hexachlorobutadiene (HCBD) + HCBD ¹³ C ₄ |
| 4 | 9.71 | Pentachlorobenzene |
| 5 | 10.78 | alpha-HCH |
| 6 | 10.88 | Hexachlorobenzene (HBH) + HCB ¹³ C ₆ |
| 7 | 11.04 | beta-HCH |
| 8 | 11.14 | gamma-HCH |
| 9 | 11.37 | delta-HCH |
| 10 | 11.76 | Alachlore D13 |
| 11 | 11.8 | Alachlore |
| 12 | 12.29 | Aldrin |
| 13 | 12.6 | Isodrin |
| 14 | 13.03 | Endosulfan I (alpha) + Endosulfan alpha D4 |
| 15 | 13.17 | pp'-DDE |
| 16 | 13.31 | Dieldrin |
| 17 | 13.53 | Endrin |
| 18 | 13.58 | pp'-DDD |
| 19 | 13.6 | Endosulfan II (beta) |
| 20 | 13.64 | op'-DDT |
| 21 | 13.95 | pp'-DDT |

Table 6. Results from Tap Water Samples

| Substance | Recovery (%) | | | Mean (%) | RSD(%) | Acceptance Criteria (% - %) | Passed Failed |
|-------------------------|--------------|-------|-----------------|----------|--------|-----------------------------|------------------|
| | Blank | Spike | Spike Duplicate | | | | |
| Alachlore | ND | 107.2 | 105.3 | 106.3 | 1.3 | 70 -120 | Passed |
| Aldrin | ND | 97.3 | 96.2 | 96.7 | 0.8 | 70 -120 | Passed |
| Dieldrin | ND | 99.7 | 102.3 | 101.0 | 1.8 | 70 -120 | Passed |
| Endrin | ND | 108.4 | 109.9 | 109.2 | 1.0 | 70 -120 | Passed |
| Isodrin | ND | 103.8 | 98.3 | 101.0 | 3.9 | 70 -120 | Passed |
| op'-DDT | ND | 111.2 | 112.7 | 112.0 | 0.9 | 70 -120 | Passed |
| pp'-DDT | ND | 110.6 | 115.8 | 113.2 | 3.3 | 70 -120 | Passed |
| pp'-DDD | ND | 101.9 | 103.7 | 102.8 | 1.3 | 70 -120 | Passed |
| pp'-DDE | ND | 107.2 | 106.8 | 107.0 | 0.2 | 70 -120 | Passed |
| Hexachlorobenzene | ND | 107.8 | 109.1 | 108.5 | 0.8 | 70 -120 | Passed |
| Hexachlorobutadiene | ND | 105.1 | 107.5 | 106.3 | 1.6 | 70 -120 | Passed |
| alpha-HCH | ND | 107.4 | 104.9 | 106.2 | 1.7 | 70 -120 | Passed |
| beta-HCH | ND | 113.7 | 109.3 | 111.5 | 2.8 | 70 -120 | Passed |
| delta-HCH | ND | 110.6 | 107.3 | 108.9 | 2.2 | 70 -120 | Passed |
| gamma-HCH | ND | 111.6 | 107.2 | 109.4 | 2.8 | 70 -120 | Passed |
| Pentachlorobenzene | ND | 109.0 | 111.4 | 110.2 | 1.6 | 70 -120 | Passed |
| 1,2,3-TCB | ND | 107.1 | 107.1 | 107.1 | 0.1 | 70 -120 | Passed |
| 1,2,4-TCB | ND | 110.8 | 111.0 | 110.9 | 0.1 | 70 -120 | Passed |
| 1,3,5-TCB | ND | 102.6 | 102.6 | 102.6 | 0.0 | 70 -120 | Passed |
| Endosulfan-I (alpha) | ND | 113.5 | 116.2 | 114.9 | 1.7 | 70 -120 | Passed |
| Endosulfan-II (beta) | ND | 106.3 | 112.4 | 109.3 | 3.9 | 70 -120 | Passed |
| Alachlore D13 | - | 105.3 | 98.1 | 101.7 | 5.0 | 70 -120 | Passed |
| HCB 13C6 | - | 103.0 | 95.0 | 99.0 | 5.7 | 70 -120 | Passed |
| HCBD 13C4 | - | 105.3 | 105.2 | 105.2 | 0.0 | 70 -120 | Passed |
| Endosulfan-I D4 (alpha) | - | 99.6 | 89.1 | 94.4 | 7.9 | 70 -120 | Passed |

Conclusion

This work demonstrates the successful performance of automated SPE with SPE disks for the extraction of a OCPs mixture. Automated SPE using SPE disks complies with method requirements (European Standard EN 16693:2015) and provides excellent recoveries between 70 and 120 %. Automated SPE using SPE disk therefore meets the Water Framework Directive requirements.

References

1. Directive 2013/39/EU of the European Parliament and of the Council.
2. European Standard EN 16693:2015.

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