

Organochlorine Pesticides by GCxGC-ECD

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1. Introduction

United States Environmental Protection Agency Method (EPA) 8081 uses gas chromatography–electron capture detection (GC-ECD) to quantify organochlorine pesticides (OCPs) in extracts from solid and liquid matrices. The method includes a parallel dual-column option in which the GC is configured with one injection port, directing extracts to two different GC columns that terminate in two ECDs. This configuration helps to confirm quantified values that are obtained when a non-specific detector such as the ECD is used. Conversely, biases that arise due to pesticide coelutions with interferences are illuminated.

A relatively new way to solve separation problems is to use comprehensive two-dimensional GC (GCxGC). GCxGC is a way to increase peak capacity by applying two independent separations to a sample in one analysis with one detector. Typically, GCxGC involves a serial column configuration (differing phases) separated by a thermal modulator. A separation is performed on the first column, and then effluent from the first column is continually (and quickly) focused and "injected" onto the second column. By keeping the second column short, a series of high-speed chromatograms are generated, and the first column separation can be maintained. Separation results can be plotted as a retention plane (column 1 time x column 2 time), also known as a contour plot. By using GCxGC, the chances for coeluting interferences are reduced, and an analogy can be drawn between GCxGC and parallel dual-column analysis.

This application note describes a comparison of OCP results obtained from a parallel dual-column GC-ECD method and a GCxGC-ECD method. Soil and water extracts were quantified using the external standard method.

Standards

The standards were obtained from Restek and contained the following OCPs: aldrin, alpha-chlordane, alpha-hexachlorocyclohexane (HCH), beta-HCH, DDD, DDE, DDT, delta-HCH, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, gamma-chlordane, gamma-HCH, heptachlor, heptachlor epoxide, and methoxychlor. Decachlorobiphenyl (209) and tetrachloro-m-xylene (TCMX) were also in the standards and serve as surrogate compounds for method 8081.

The standards were diluted in hexane to achieve the following concentrations (in pg/ μ L) for calibration curves.

- HCHs, heptachlors, aldrin, chlordanes, endosulfans (5, 10, 20, 40, 80)
- 209, TCMX, dieldrin, endrins, endosulfan sulfate, DDT compounds (10, 20, 40, 80, 160)
- Methoxychlor (50, 100, 200, 400, 800)

Samples

Soil and water extracts were obtained from Severn Trent Laboratories, STL Burlington, in Colchester, Vermont. Soil was extracted using EPA Method 3550, ultrasonic extraction. Additionally, gel permeation chromatography was applied to the soil extracts using EPA Method 3640. Water was liquid-liquid extracted in a separatory funnel according to EPA Method 3510. Both soil and water extracts were subjected to Florisil column clean-up following EPA Method 3620.

2. Experimental Conditions

Parallel Dual-Column GC-ECD

Column 1:

30 m x 0.32 mm x 0.50 μ m Rtx-CLPesticide (Restek)

Column 2:

30 m x 0.32 mm x 0.42 μ m Rtx-CLPesticidell (Restek)

Carrier:

Hydrogen at 2.8 mL/minute, constant flow

Injection:

2 μ L direct at 200°C

Oven Program:

120°C (1 minute), 16°/minute to 210°, 13°/minute to 245°, 12.5°/minute to 300° (4 minute)

Total Run Time: 17 minutes

Detector:

ECD, 300°C, argon/methane makeup gas at 140 mL/minute

LECO GCxGC-ECD

Agilent 6890 GC-ECD equipped with a LECO Quad Jet—Dual-Stage Thermal Modulator

Column 1:

9 m x 0.18 mm x 0.20 μ m Rtx-5 (Restek)

Column 2:

1 m x 0.18 mm x 0.20 μ m Rtx-200 (Restek)

Carrier:

Helium at 2 mL/minute, constant flow

Injection:

1 μ L split at 250°C, split ratio 50:1

Oven 1 Program:

50°C (0.2 minute), 30°/minute to 140°, 5°/minute to 250°

Oven 2 Program:

50°C offset from oven 1

Modulation:

Temperature offset 30°C from oven 1, time 6 seconds

Total Run Time: 25.2 minutes

Detector:

ECD, 325°C, N₂ makeup gas at 148 mL/minute, 50 Hz

Data Processing

LECO ChromaTOF[®] software was used to automatically peak find and quantify organochlorine pesticides analyzed with GCxGC-ECD.

3. Results

Analysis of Standards with GCxGC-ECD

Figure 1 is a contour plot of an OCP standard analyzed with GCxGC-ECD. Note that the X-axis represents the first dimension retention time, and the Y-axis shows the second dimension retention time (the actual retention times are recorded in Table 1). Peak intensity, as defined by detector response, is represented by a color scheme from blue (zero, or baseline detector response) to red (most intense response). Each "spot" represents a peak (and pesticide). Figure 2 demonstrates the power of GCxGC by showing how beta- and gamma-HCH, which coelute on Rtx-5 (in the first dimension), are separated by Rtx-200 in the second dimension.

Another way to visualize GCxGC data is with a surface plot (illustrated in Figure 3). In this plot, the Z-axis represents peak intensity (as defined by ECD response).



Figure 1. Contour plot (GCxGC chromatogram) of organochlorine pesticide mix. Note the separation of compounds in two dimensions with the Rtx-5 separation (and retention time) on the X-axis, and the Rtx-200 separation occurring along the Y-axis.

Table 1. Retention times (RTs) for OCPs via GCxGC-ECD.

Pesticide	RT 1 sec (Rtx-5)	RT 2 sec (Rtx-200)
Tetrachloro -m-xylene	294	1.90
alpha-HCH	342	2.44
beta-HCH	378	2.96
gamma-HCH	384	2.66
delta-HCH	420	2.94
Heptachlor	480	2.52
Aldrin	534	2.58
Heptachlor epoxide	606	3.16
gamma-Chlordane	648	2.96
Endosulfan I	672	3.32
alpha-Chlordane	678	2.96
Dieldrin	720	3.34
4,4'-DDE	732	2.72
Endrin	756	3.46
Endosulfan II	780	3.78
4,4'-DDD	810	3.18
Endrin aldehyde	816	4.50
Endosulfan sulfate	864	5.20
4,4'-DDT	882	2.96
Endrin ketone	942	4.68
Methoxychlor	1008	2.82
Decachlorobiphenyl	1320	2.62

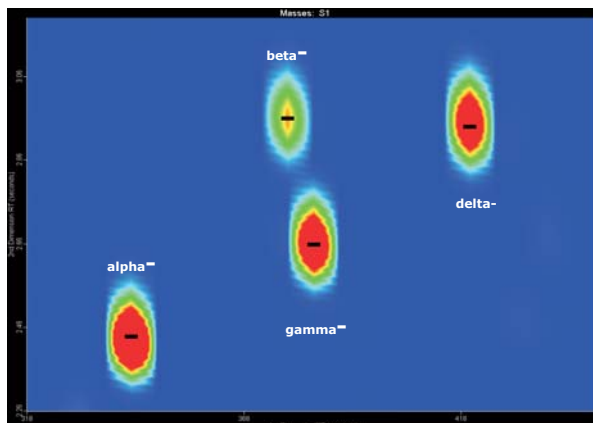


Figure 2. Separation of HCHs with GCxGC-ECD. Beta- and gamma-HCH coelute in the first dimension on Rtx-5, but are easily separated in the second dimension with Rtx-200.

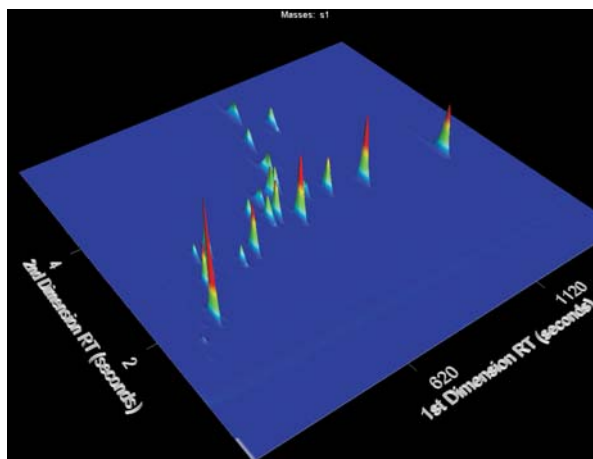


Figure 3. Surface plot of organochlorine pesticide mix analyzed with GCxGC-ECD. The first dimension retention time is for the Rtx-5 separation, and the second dimension retention time is for the Rtx-200 separation. In addition, there is a Z-axis which represents ECD response for the pesticides.

Calibration with GCxGC-ECD

Calibration curves were established for GCxGC-ECD by the external standard method using the standard concentrations listed above. Example calibration curves for one of the better ECD responding compounds (gamma-HCH or Lindane), and the worst responding compound (Methoxychlor) are shown in Figures 4 and 5.

Although all of the GCxGC-ECD quantifications for the samples were performed using calibrations established based on concentrations listed in the Standards section, it is possible to go much lower due to the extreme sensitivity afforded by the ECD and the sensitivity enhancement achieved by the focusing effect of GCxGC. Table 2 lists low points and the correlation coefficients when the curve is further extended to even lower OCP concentrations. Again, it is important to point out that since a split injection was used, some of the actual amounts on column (and to the detector) are very low. For example, a 0.5 pg/ μ L standard represents 10 fg (!) on column.

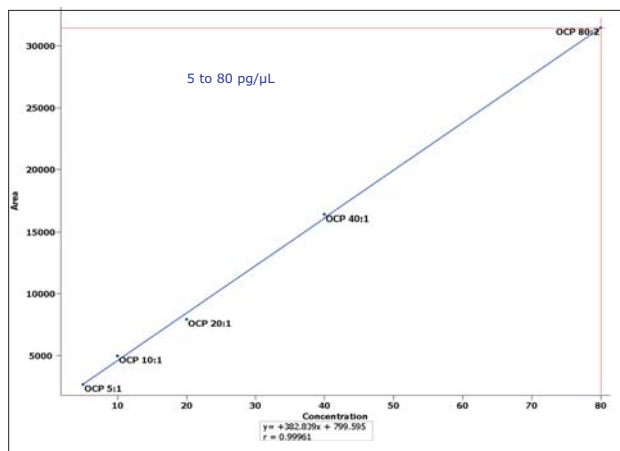


Figure 4. GCxGC-ECD calibration curve for gamma-HCH (Lindane). Note that the concentrations are in pg/μL (listed as black numbers by the points, e.g. 5:1 = 5 pg/μL), but due to split injection at a ratio of 50:1, the low point represents only 0.1 pg on column.

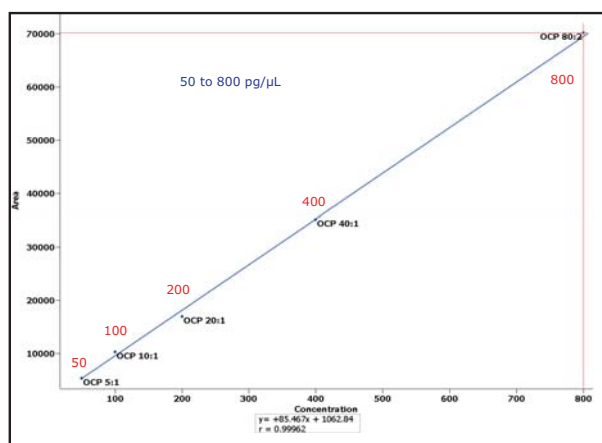


Figure 5. GCxGC-ECD calibration curve for Methoxychlor from 50 to 800 pg/μL. Note that the concentrations are in pg/μL (listed in red near the points on the curve), but due to split injection at a ratio of 50:1, the low point represents only 1 pg on column.

Quantitative Comparison of GCxGC-ECD and Parallel Dual-Column GC-ECD

Tables 3 and 4 compare GCxGC-ECD and GC-ECD results for water and soil laboratory control spikes. These samples were uncontaminated with OCPs (and presumably other halogenated components) previous to the spikes so they represent a good foundation for comparing results. As can be seen, the concentration values compare nicely between the two techniques.

Table 2. Calibration curves extended to values lower than those used to quantify samples for OCPs.

Pesticide	Low point (pg/μL)	Correlation Coefficient
Tetrachloro-m-xylene	1	0.9983
alpha-HCH	0.5	0.9996
beta-HCH	0.5	0.9995
gamma-HCH	0.5	0.9995
delta-HCH	0.5	0.9994
Heptachlor	0.5	0.9993
Aldrin	0.5	0.9994
Heptachlor epoxide	0.5	0.9995
gamma-Chlordane	0.5	0.9997
Endosulfan I	0.5	0.9997
alpha-Chlordane	0.5	0.9996
Dieldrin	1	0.9996
4,4'-DDE	1	0.9996
Endrin	1	0.9995
Endosulfan II	1	0.9996
4,4'-DDD	1	0.9996
Endrin aldehyde	1	0.9987
Endosulfan sulfate	1	0.9995
4,4'-DDT	1	0.9996
Endrin ketone	1	0.9994
Methoxychlor	5	0.9997
Decachlorobiphenyl	1	0.9996

Table 3. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/μL) for a water laboratory control spike extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	34.7	41.5
alpha-HCH	ND	ND
beta-HCH	ND	ND
gamma-HCH	57.9	58.9
delta-HCH	ND	ND
Heptachlor	54.5	61.7
Aldrin	49.6	62.8
Heptachlor epoxide	ND	ND
gamma-Chlordane	ND	ND
Endosulfan I	ND	ND
alpha-Chlordane	ND	ND
Dieldrin	115	126
4,4'-DDE	ND	ND
Endrin	116	141
Endosulfan II	ND	ND
4,4'-DDD	ND	ND
Endrin aldehyde	ND	ND
Endosulfan sulfate	ND	ND
4,4'-DDT	110	134
Endrin ketone	ND	ND
Methoxychlor	ND	ND
Decachlorobiphenyl	40.0	41.0

ND = not detected.

Table 4. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/ μ L) for a soil laboratory control spike extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	31.0	35.8
alpha-HCH	21.6	19.3
beta-HCH	22.3	20.3
gamma-HCH	21.7	19.6
delta-HCH	21.8	19.6
Heptachlor	20.8	19.2
Aldrin	19.0	18.9
Heptachlor epoxide	21.7	18.9
gamma-Chlordane	20.3	18.4
Endosulfan I	19.6	18.1
alpha-Chlordane	19.6	18.7
Dieldrin	41.9	38.7
4,4'-DDE	40.6	37.1
Endrin	42.3	38.8
Endosulfan II	41.4	38.0
4,4'-DDD	41.4	40.3
Endrin aldehyde	30.3	22.4
Endosulfan sulfate	40.4	43.2
4,4'-DDT	40.0	39.3
Endrin ketone	42.4	43.2
Methoxychlor	195	218
Decachlorobiphenyl	33.7	39.3

ND = not detected.

In Table 5, GCxGC-ECD and GC-ECD results are compared for a "real world" water extract. To start with, looking at the surrogate results (tetrachloro-m-xylene and decachlorobiphenyl) for each method is a good way to gauge the results. As might be expected with water samples, which in general are less complex and less subject to interferences versus soil samples, the numbers are in good agreement.

For "real world" soil extracts, differences start to show between GCxGC-ECD and parallel dual-column GC-ECD results. In Table 6, highlighted in red are some of the more dramatic concentration differentials seen for a soil extract. Especially note the highly biased value for gamma-HCH with GC-ECD. Based on gas chromatography-time-of-flight mass spectrometry (GC-TOFMS) analysis, this sample was seen to contain polychlorinated biphenyls (PCBs), and that is what is likely causing the high gamma-HCH value for GC-ECD. Interestingly, neither column used in the parallel dual-column work (Rtx-CLPesticides and Rtx-CLPesticidesII) provided an unbiased gamma-HCH concentration.

Table 5. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/ μ L) for a "real world" water extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	21.4	19.8
alpha-HCH	ND	ND
beta-HCH	10.5	8.91
gamma-HCH	49.9	39.0
delta-HCH	ND	ND
Heptachlor	39.2	31.1
Aldrin	21.7	21.5
Heptachlor epoxide	ND	ND
gamma-Chlordane	35.9	33.4
Endosulfan I	ND	ND
alpha-Chlordane	59.1	61.0
Dieldrin	13.9	10.1
4,4'-DDE	35.1	32.6
Endrin	61.0	54.2
Endosulfan II	ND	ND
4,4'-DDD	ND	ND
Endrin aldehyde	ND	ND
Endosulfan sulfate	36.4	31.5
4,4'-DDT	47.9	43.3
Endrin ketone	ND	ND
Methoxychlor	134	114
Decachlorobiphenyl	24.0	20.3

ND = not detected.

Table 6. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/ μ L) for a "real world" soil extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	31.8	25.9
alpha-HCH	ND	15.9
beta-HCH	ND	ND
gamma-HCH	ND	64.9
delta-HCH	ND	9.19
Heptachlor	1.44	ND
Aldrin	ND	ND
Heptachlor epoxide	20.3	10.3
gamma-Chlordane	78.5	57.6
Endosulfan I	ND	ND
alpha-Chlordane	68.3	66.3
Dieldrin	10.8	6.92
4,4'-DDE	30.8	20.2
Endrin	0.27	27.8
Endosulfan II	ND	ND
4,4'-DDD	60.4	22.1
Endrin aldehyde	ND	5.18
Endosulfan sulfate	ND	ND
4,4'-DDT	211	145
Endrin ketone	ND	12.7
Methoxychlor	11.8	ND
Decachlorobiphenyl	35.9	34.2

ND = not detected.

In another example (Table 7) where the soil extract had very high concentrations of PCBs, as confirmed by GC-TOFMS, the biases for GC-ECD are even more striking (again highlighted in red).

Table 7. Comparison of GCxGC-ECD and parallel dual-column GC-ECD results (pg/μL) for a "real world" soil extract.

Pesticide	GCxGC-ECD	GC-ECD
Tetrachloro-m-xylene	2.06	1.52
alpha-HCH	ND	4.96
beta-HCH	ND	3.94
gamma-HCH	154	118
delta-HCH	ND	42.1
Heptachlor	28.1	13.1
Aldrin	26.4	70.0
Heptachlor epoxide	ND	42.2
gamma-Chlordane	3.26	ND
Endosulfan I	ND	ND
alpha-Chlordane	ND	99.4
Dieldrin	1.45	12.6
4,4'-DDE	124	199
Endrin	ND	216
Endosulfan II	ND	28.4
4,4'-DDD	ND	121
Endrin aldehyde	ND	49.1
Endosulfan sulfate	ND	ND
4,4'-DDT	70.9	480
Endrin ketone	ND	ND
Methoxychlor	60.4	ND
Decachlorobiphenyl	2.80	2.75

ND = not detected.

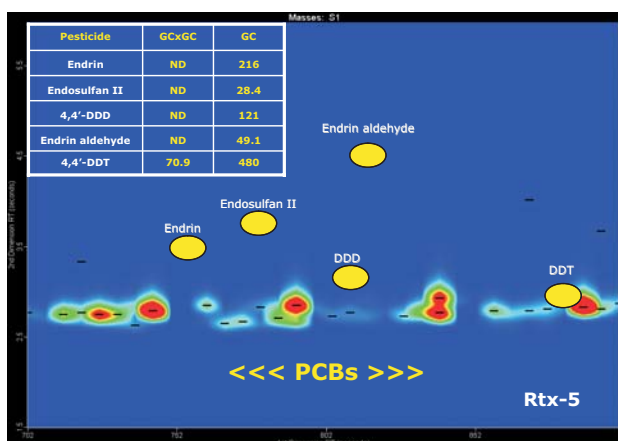


Figure 6. Zoomed in contour plot of a "real world" soil extract showing PCBs eluting along a relatively straight line in the first dimension. The OCPs (with their chromatographic places marked by yellow ovals), except for DDT, are moved off of the X-axis by the Rtx-200 column, and are less prone to interference (and high quantitative bias), as seen in the inset table results.

The reason for the less biased performance of GCxGC-ECD can be deduced from the contour plot shown in Figure 6. The PCBs tend to elute in a relatively straight line along the X-axis since they are not as significantly retained on Rtx-200 as the pesticides marked with yellow ovals in the figure. Moving the pesticides away from this chromatographic line with the second dimension separation leads to less chance of an erroneously high quantitation value for an OCP.

4. Conclusions

GCxGC is a powerful way to reduce the possibility of quantification bias when using a non-specific detector such as an ECD. As shown in this application note, it may be better than parallel dual-column analysis (while still providing a dual-column approach), for the organochlorine pesticides of EPA Method 8081, especially when the samples contain PCBs.

Calibrations (ECD responses) are linear, even across relatively wide concentration ranges. Due to the focusing effect of GCxGC, where peaks are sharpened close to the detector, sensitivity is improved, which allows detection of low femtogram levels of many OCPs.

Split injections of dirty samples, possible due to the elevated sensitivity afforded when using GCxGC-ECD, may lead to less downtime due to injector and column maintenance.

5. Acknowledgment

Jennifer Clements and Bryce Stearns at STL Burlington kindly provided the sample extracts, the GC-ECD results, and also helped with data interpretation.

6. References

United States Environmental Protection Agency, Test Methods: SW-846 on-line;
http://www.OLE_LINK1.epa.gov/epaoswer/hazwaste/test/main.htm

