

QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS USING GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION

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ABSTRACT

Chlorinated hydrocarbons, including polychlorinated biphenyls and pesticides, are quantitatively determined using gas chromatograph/electron capture detector. This method unambiguously detects low concentration of chlorinated hydrocarbons in tissues and sediments.

1.0 INTRODUCTION

A gas chromatograph/electron capture detector (GC/ECD) coupled to two capillary columns is used to resolve, detect and quantitate chlorinated hydrocarbons (polychlorinated biphenyls and pesticides) in tissues and sediments at parts per billion levels. Samples are injected into a temperature-programmed GC/ECD, operated in splitless mode. The capillary columns are DB-5 (30 m x 0.25 mm ID and 25 μm film thickness) and DB-17HT (30 m x 0.25 mm ID and 0.15 μm film thickness). The DB-17HT column is used for analyte confirmation. The data acquisition system continuously acquires and stores all data analyses.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Gas chromatograph, split/splitless injection port and electronic pressure control, dual electron capture detectors
- Data acquisition system, capable of continuous acquisition and storage of all data during analysis
- Autosampler, capable of making 1 to 5 μL injections
- Capillary columns, J&W DB-5[®] (30 m x 0.25 mm ID and 0.25 μm film thickness) or equivalent, and J&W DB-17HT[®] (30 m X 0.25 mm ID and 0.15 μm film thickness) or equivalent

- Micropipetters, calibrated, 1% accuracy, disposable tips

2.2 REAGENTS

- Hexane, pesticide grade or equivalent
- Helium, 99.8% purity
- 95% Argon/5% Methane, 99.8% purity

2.3 STANDARDS

2.3.1 *Surrogate Spiking Solution*

A surrogate spiking solution is prepared from a commercially available solution that is diluted with hexane to a concentration of 1,000 pg/ μ L. The surrogate spiking solution includes 4,4'-dibromooctofluorobiphenyl (DBOFB), 2,2',4,5',6 pentachlorobiphenyl (PCB 103), and 2,2',3,3',4,5,5'6 octochlorobiphenyl (PCB 198). Surrogate solution is added to all samples and all quality control samples prior to extraction. Surrogate compounds are resolved from, but elute in close proximity to the analytes of interest. The recovery of PCB 103 is used to correct analyte concentrations.

2.3.2 *Internal Standard Solution*

The internal standard solution is prepared from a commercially available solution of tetrachloro-m-xylene (TCMX) diluted with hexane to a final concentration of 1,000 pg/ μ L. The internal standard compound is resolved from, but elutes in close proximity to the analytes of interest. The internal standard solution is added to all samples and quality control samples just prior to analysis. Internal standards are used to calculate analyte concentrations.

2.3.3 *Matrix Spiking Solution*

To prepare the matrix spiking solution, a certified solution containing analytes of interest is purchased from commercial vendors and diluted with hexane (Table 1). The matrix spike solution is diluted to approximately 10 times the MDL and is added to all matrix spike samples.

2.3.4 *Calibration Solution*

Calibrations solutions are prepared at 5 concentrations ranging from approximately 5 to 200 pg/ μ L (Table 2) by diluting a commercially prepared solution containing the analytes of interest.

Table 1. Chlorinated Hydrocarbons Contained in Matrix Spike Solution at 40 pg/ μ l.

<u>Pesticides</u>	<u>PCBs</u>
1,2,4,5-Tetrachlorobenzene	PCB 8
1,2,3,4-Tetrachlorobenzene	PCB 18
Pentachlorobenzene	PCB 28
Pentachloroanisole	PCB 44
Chlorpyrifos	PCB 52
Hexachlorobenzene	PCB 66
α -HCH	PCB 101
β -HCH	PCB 105
γ -HCH (Lindane)	PCB 118
δ -HCH	PCB 128
Heptachlor	PCB 138
Heptachlor epoxide	PCB 153
α -Chlordane (cis-)	PCB 170
γ -Chlordane (trans-)	PCB 180
Trans-Nonachlor	PCB 187
Cis-Nonachlor	PCB 195
Aldrin	PCB 206
Dieldrin	PCB 209
Endrin	
Mirex	
2,4' DDE	
4,4' DDE	
2,4' DDD	
4,4' DDD	
2,4' DDT	
4,4' DDT	
Endosulfan II	
Oxychlordane	
Endosulfan Sulfate	

Table 2. Chlorinated Hydrocarbons Contained in Calibration Solutions and their Concentrations.

Compounds Contained in Calibration Solutions	Level 1 (pg/ml)	Level 2 (pg/ml)	Level 3 (pg/ml)	Level 4 (pg/ml)	Level 5 (pg/ml)
<u>Internal Standard</u>					
TCMX	100	100	100	100	100
<u>Surrogates</u>					
DBOFB	5	20	40	80	200
PCB 103	5	20	40	80	200
PCB 198	5	20	40	80	200
<u>Analytes</u>					
1,2,4,5-Tetrachlorobenzene	5	20	40	80	200
1,2,3,4-Tetrachlorobenzene	5	20	40	80	200
Pentachlorobenzene	5	20	40	80	200
Pentachloroanisole	5	20	40	80	200
Chlorpyrifos	5	20	40	80	200
Hexachlorobenzene	5	20	40	80	200
α-HCH	5	20	40	80	200
β-HCH	5	20	40	80	200
γ-HCH	5	20	40	80	200
δ-HCH	5	20	40	80	200
Heptachlor	5	20	40	80	200
Heptachlor epoxide	5	20	40	80	200
Oxychlordane	5	20	40	80	200
α-Chlordane (cis-)	5	20	40	80	200
γ-Chlordane (trans-)	5	20	40	80	200
Trans-Nonachlor	5	20	40	80	200
Cis-Nonachlor	5	20	40	80	200
Aldrin	5	20	40	80	200
Dieldrin	5	20	40	80	200
Endrin	5	20	40	80	200
Mirex	5	20	40	80	200
2,4' DDE	5	20	40	80	200
4,4' DDE	5	20	40	80	200
2,4' DDD	5	20	40	80	200
4,4' DDD	5	20	40	80	200
2,4' DDT	5	20	40	80	200
4,4' DDT	5	20	40	80	200
Endosulfan II	5	20	40	80	200
Endosulfan Sulfate	5	20	40	80	200
PCB 8	5	20	40	80	200
PCB 18	5	20	40	80	200
PCB 28	5	20	40	80	200
PCB 44	5	20	40	80	200
PCB 52	5	20	40	80	200
PCB 66	5	20	40	80	200
PCB 101	5	20	40	80	200
PCB 105	5	20	40	80	200
PCB 118	5	20	40	80	200
PCB 128	5	20	40	80	200
PCB 138	5	20	40	80	200
PCB 153	5	20	40	80	200
PCB 170	5	20	40	80	200
PCB 180	5	20	40	80	200
PCB 187	5	20	40	80	200
PCB 195	5	20	40	80	200
PCB 206	5	20	40	80	200
PCB 209	5	20	40	80	200

3.0 QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS BY GC/ECD

3.1 CALIBRATION

An ECD exhibits limited linearity, particularly for low concentrations. Consequently, a calibration must be established for each analytical run. An analytical run consists of samples and 5 calibration standards (approximately 5 to 200 pg/ μ L or 5 to 200 ng/mL) that are interspersed throughout the run. A calibration curve is established by analyzing the 5 interspersed calibration standards and fitting the data to the following quadratic equation.

$$x = \frac{-b_1 + \sqrt{b_1^2 - 4b_2(b_0 - Y)}}{2b_2}$$

Where:

X= the concentration of the analyte (ng/ml)

Y= the ratio of the area of the analyte to the area of the internal standard multiplied by the amount of the internal standard (ng)

b_2, b_1, b_0 = the coefficients for the quadratic equation

The data generated for each analyte in the calibration standards are subjected to the method of least squares to determine the coefficients for the corresponding quadratic equation. Each analyte has different coefficients based on the relative response of the analyte compared to the internal standard, and as a function of the amount of the analyte. The injected concentration of the internal standard analyte is held constant for each set of calibration standards. In order for the calibration to be valid, each analyte must have a correlation coefficient greater than 0.997.

3.2 GC/ECD ANALYSIS

Sample analyses are completed only if the calibration meets previously described criteria. Samples are analyzed in analytical sets that consist of standards, samples and quality control samples. Quality control samples are method blanks, laboratory duplicates, blank spikes, matrix spikes and standard reference materials. The type and number of quality control samples depend upon client requests and material availability. An autosampler is used to inject 1 or 5 μ L of all samples, standards and QC samples into the capillary column of the GC using the following instrument conditions. Slight modifications may be necessary depending upon the analysis.

Inlet: Splitless
Carrier gas: Helium, 1 mL/min

Temperatures:
Injection port: 275°C
Detector: 325°C

Oven program:
Initial oven temp: 100°C
Initial hold time: 1 minute
Ramp rate: 5°C/min to 140°C
Hold time: 1 minute
Ramp rate: 1.5°C/min to 250°C
Hold time: 1 minute
Final oven rate: 10°C/min to 300°C
Final hold time: 5 minutes

3.3 ANALYTE IDENTIFICATION

The retention time of a sample analyte must fall within 15 seconds of the retention time for the analyte in a calibration standard or a retention index solution.

Interferences may exist which limits the analyst's ability to quantify a peak or peaks correctly. Data may be reported but appropriately qualified to indicate interference or are re-analyzed.

4.0 QUANTITATION CALCULATIONS

The concentration (C) of each target analyte in the sample (ng/g) is calculated using the following equation:

$$C = \left(\frac{X}{W} \right) (V_e DF)$$

Where:

V_e = the final volume of the extract (mL)

X = the concentration of the analyte (ng/mL) as found from solving the quadratic equation

W = the sample weight (g)

DF = the dilution factor

$$DF = \frac{\text{Volume of Extract (uL)}}{\text{Volume of extract used to make dilution (uL)}}$$

Analyte concentrations are reported as corrected for surrogate recoveries. Percent surrogate recoveries (SU_{recovery}) for each surrogate are calculated using the following equation:

$$SU_{\text{Recovery}} = \frac{C_{\text{ESU}}}{C_{\text{SU}}} \times 100$$

Where:

C_{ESU} = calculated surrogate concentration in the extract

C_{SU} = known concentration of surrogate added to extract

Analyte concentration corrections ($C_{\text{corrected}}$) for surrogate recovery are calculated using the following equation:

$$C_{\text{Corrected}} = \frac{C}{SU_{\text{Recovery}}} \times 100$$

5.0 QUALITY CONTROL

The interspersed calibration must pass established criteria before sample analysis can be completed.

An acceptable method blank analysis may not contain more than two target analytes at concentrations three times greater than the MDL. This criterion does not apply if the analytes detected in the method blank are not detected in the associated samples or if the sample analyte concentrations are 10 times greater than the blank analyte concentrations. If the method blank exceeds these criteria then the analytical procedure is not in control. The source of the contamination must be investigated, and corrective measures taken and documented before further sample analysis occurs.

All samples and quality control samples are spiked with chlorinated surrogate compounds prior to extraction. The surrogate compounds evaluate sample matrix effects and analytical efficiencies associated with sample preparation and analysis. The recovery of the surrogate compounds is monitored in each sample and quality control sample. The laboratory will take corrective action if the average surrogate recovery is less than 50% or greater than 150%. The following corrective action will be taken if the above criteria are not met:

- 1) Calculations are checked to assure that no errors have been made.
- 2) The internal standard and surrogate solutions are checked for degradation, contamination, etc., and instrument performance is checked.
- 3) If the upper control limit is exceeded for a surrogate, and the instrument calibration and surrogate standard concentration are in control, it is concluded that an interference specific to the surrogate was present that resulted in high recovery and that this

interference does not affect the quantitation of other target compounds. The presence of this type of interference is confirmed by evaluation of chromatographic peak shapes. If analytes are surrogate corrected, another surrogate eluting in close proximity may be used to calculate surrogate corrected analyte concentrations and the change is appropriately documented.

- 4) If the surrogate cannot be measured because only a portion of the sample is analyzed (such as sample dilution) then no corrective action is required. The surrogate recovery is appropriately qualified.
- 5) If the steps above fail to reveal a problem, the extract is re-analyzed, if sufficient material is available. If the sample was completely consumed, the data will be reported but designated as outside the quality control criteria..

The average recovery for all compounds in a blank spike or valid matrix-spike must fall between 80% and 120%. No more than two individual analyte recoveries in a blank spike or valid matrix spike can exceed 40-120%, with the exception of chlorpyrifos and endosulfan sulfate. A valid matrix-spike is a matrix-spike where the amount of analyte added is at least as much as was present in the sample originally. If the matrix spike is valid and the QC criteria are not met then the quality control sample failing the criteria will be re-analyzed. If the re-analyzed spike meets the criteria then the data for the re-analyzed quality control sample are reported. If the analytes that are in violation are not present in the samples analyzed with this extraction batch, the violation is noted but no action is required. If upon re-analysis, quality control criteria are still violated, the entire batch of samples is re-extracted and re-analyzed, if deemed necessary. If sufficient sample is unavailable for the re-extraction of the matrix-spike, a blank-spike may be analyzed. The acceptable average RPD for a valid matrix spike/matrix spike duplicate or blank spike/blank spike duplicate pair is 30%. No more than two individual analyte RPDs may exceed 35%.

The average acceptable RPD between the duplicate and original sample, for analytes greater than 10 times the concentration of the MDL, is 30%. No more than two individual analyte RPDs can exceed 35%.

The average RPD for target compounds in a SRM should not exceed 30% of the upper and lower bounds of the mean certified values. No more than two target analytes should deviate more than 35% from the upper or lower bounds of the mean certified values.

The method detection limit (MDL) is determined following the procedures outlined in Federal Register (1984), Vol. 49, No. 209: 198-199.