Identification and quantification of organochlorine pesticides by GC-ECD in environmental matrices

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Abstract. An analytical method for the determination of organochlorine pesticides (OCPs) (α-HCH, β-HCH, γ-HCH (Lindane), δ-HCH, Aldrin, Dieldrin, Endrin, DDT, DDD, DDE, Heptachlor, Chlordane, Endosulfan I and Endosulfan II) in environmental samples (wastewater, soil) uses liquid phase extraction and gas chromatography with electron capture detector (GC-µECD) quantification was developed. The analytes detection was performed on an Agilent 8890B GC system using a DB5 capillary column (60 m X 0.25 mm, 0.25 µm) for the separation of the compounds. The structural and retention time confirmation for the studied compounds was done using a ThermoTSQ 8000 Evo GC-MS/MS system with the same type of column and similar operating parameters. The extraction from wastewater samples was done using hexane as non-polar solvent in a separatory funnel. For soil samples the extraction was carried out in 50 mL centrifuge tubes using acetone as polar solvent and hexane as non-polar solvent. The repeatability and the intermediate precision of the GC-µECD method were determined. The obtained values demonstrate that the method is accurate with RSD less than15% and can be successfully applied for studied matrices.

Keywords: pesticides, GC-µECD, GC-MS/MS, wastewater, extraction

1. Introduction

Organochlorine pesticides (OCP) are chemical compounds used in the agricultural practice as insecticides and herbicides to protect the crops and the seeds against harmful pests as well as to increase the crop yield (Donald et al 2016). Other uses of OCPs include cattle disinfestation, household insecticides, combating the spread of insect carried dieseases, such as malaria and typhoid fever (Vos et al 2000) and treatment of wooden and textile materials.

As a class of chemical compounds, OCPs are characterised by robust and stable molecules which allow them to persist in nature for long periods of time either in water or soil. This persistance also allows for displacement from the place of initial aplication via groundwater, streams, rivers and even dust particles. Another chemical characteristic of this class is their lipophilicity, meaning that once ingested by animals the OCPs are stored in fatty tissues and in time can accumulate and manifest toxic effects. The primary target of these unwanted effects is the nervous system. Other affected areas are the liver, the kidneys, the immune system and the reproductory system. İn the past century there has been a number of incidents involving the uncontroled use of pesticides which resulted in great environmental damage and human suffering. All of the above considered, by the end of the twentieth century many countries classified OCPs as dangerous pollutants and legislations have been made in order to prevent further production and use (Hassaan, et al 2020, Nicolopoulou-Stamati et al 2016).

Detecting the presence of the pesticides in environmental samples is important for monitoring areas where these were produced, stored or used in the past, it is also important for finding possible unknown sources or illegal use. Various methods and techniques have been developed over time such as gas chromatography with electron capture detection (GC-ECD). Gas chromatography is a common method of chromatography used in analytical chemistry to separate and analyze compounds that can be vaporized without decomposition. The mobile phase in gas chromatography is a carrier gas, inert, or non-reactive, which passes through the stationary phase. The most commonly used gases as the mobile phase are helium, argon, nitrogen, or hydrogen gas, with high purity. The stationary phase can be represented by a microscopic layer of viscous liquid applied to an inert solid support inside a chromatographic column or a capillary made of glass or metal. The most commonly used detectors are: Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD), Electron Capture Detector (ECD/µECD), or it can be coupled with a Mass Spectrometer(MS). The ECD is used for detecting compounds with high electronegativity, such as halogenated compounds. The principle of operation is based on the presence of a radioactive material (^{63}Ni) within the detector. Electrons emitted from the radioactive material collide with the molecules of the carrier gas (usually nitrogen), resulting in the production of additional electrons. These electrons are then directed and accelerated towards a positive electrode (anode), generating a current. As analytes capable of

capturing electrons pass between the two electrodes, the current intensity is reduced in proportion to the concentration of the analytes. The Mass Spectrometer (MS) is used in tandem with gas chromatography for high-precision analyses. Unlike the ECD detector, MS provides information about the structure of the analyzed compounds based on the measurement of the mass-to-charge ratio. Its operation is based on breaking down the analyte molecule into ions, which are then isolated based on their mass-to-charge ratio and subsequently detected.

The aim of the study was to validate a method for determining organochlorine pesticides by GC-ECD technique for wastewater and soil matrices.

2. Materials and methods

2.1. The principle of the method

The determination of the content of organochlorine pesticides in wastewater samples was carried out following the international standard EN ISO 6468. For soil samples, the determination of the content of organochlorine pesticides was performed in accordance with the international standard EN ISO 10382.

The method is based on the extraction of pesticides from water, soil, and waste samples through liquid-liquid extraction, followed by their chromatographic separation on a capillary column and detection using electron capture. The method's scope of application is $1 \div 10$ ng/L for water matrices and $0.1 \div 4$ µg/kg for compounds in soil.

2.2. Verification of the equipment's performance parameters

The study was conducted on an Agilent 8890B GC-ECD chromatograph equipped with an autoinjector, split/splitless injection port, capillary column (5% phenyl-95% dimethylpolysiloxane, 60m x 0.25 mm x 0.25 µm) and electron capture detector. The operating parameters are presented in Table 1.

Table 1. The GC-ECD operating parameters

Injector	Temperature	280° C
	Total flow rate	12.2 mL/min
	Septum purge flow rate	5 mL/min
	Working mode	SPLIT
	Split ratio	5 :1
	Injection volume	1 µL
Capillary column	Constant carrier gas flow rate	1.2 mL/min
	Linear velocity	23.415 cm/sec

	Temperature program	Initial: 50ºC - hold for 1 minute - temperature ramp at 200ºC/min to 150°C - hold for 0.5 minutes - temperature ramp at 150ºC/min to 255ºC - hold for 1 minute - temperature ramp at 100ºC/min to 285ºC - hold for 4 minutes - temperature ramp at 500ºC/min to 300ºC- hold for 8 minutes.
µECD Detector	Temperature	350ºC
	Total make-up flow rate (N2 + column)	61 mL/min

3. Results and discussion

3.1. Elution order and retention time

Structural validation and elution order of the studied compounds were performed through a screening using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS). The equipment used was a Thermo TSQ 8000 Evo GC-MS/MS system, consisting of a gas chromatograph (GC 1310) coupled with a triple quadrupole mass spectrometer arranged in tandem. The chromatograms obtained from GC-ECD can be found in figure 1 while the chromatogram obtained from GC-MS/MS is presented in figure 2. Table 2 contains the elution order and retention times.
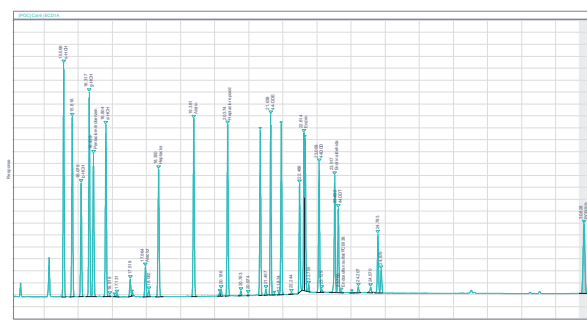

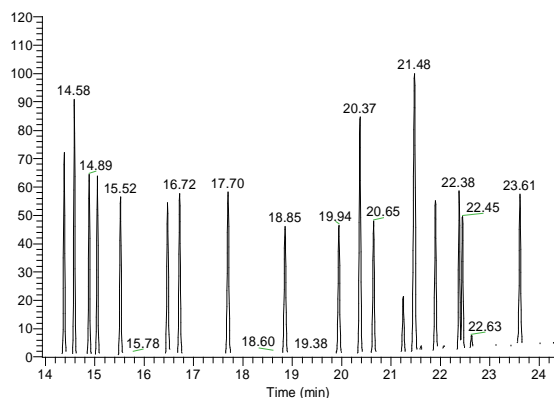**Figure 1.** The chromatogram of the OCP mixture obtained through GC-ECD

Figure 2. The chromatogram of the OCP mixture obtained through GC-MS/MS

Figure 3. Liniar regresion curve for 44'-DDT

Table 2. Elution order and retention times for the studied OCPs

Compound	Retention Time (min)
a-HCH	15.57
b-HCH	16.08
g-HCH	16.32
d-HCH	16.81
Alachlor	17.97
Heptachlor	18.35
Aldrin	19.38
Heptachlor epoxide	20.38
Endosulfan 1	21.33
44'-DDE	21.64
Dieldrin	21.95
Endrin	22.62
Endosulfan II	22.65
44'-DDD	23.06
Endrin aldehide	23.52
44'-DDT	23.62

3.2. Linearity

The calibration curves were plotted in the range between 2 µg/L and 200 µg/L. To demonstrate the linearity of the detector's response, six synthetic mixtures were prepared at concentrations of 2 µg/L, 5 µg/L, 10 µg/L, 50 µg/L, 100 µg/L, and 200 µg/L. These mixtures were prepared using a 10 mg/L stock solution of 16 OCPs mix and a 2 mg/L standard solution (SI). The stock solutions were prepared in isooctane, while the synthetic mixtures were prepared in hexane. Linear regressions were obtained, using the internal standard method, and the coefficients of determination (R^2) were greater than 0.99 (Tanase et al, 2007).
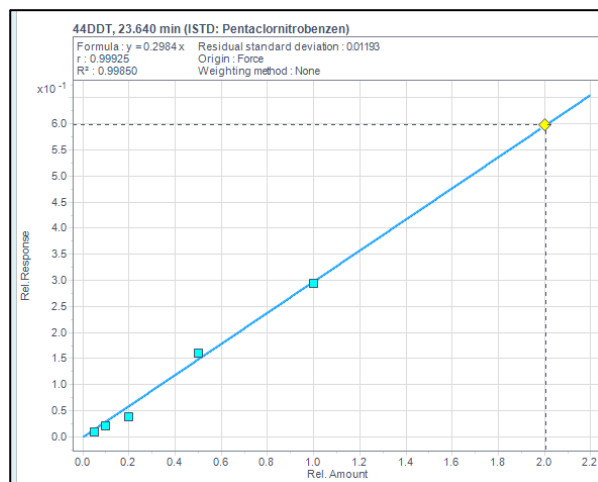
3.3. Repeatability

A solution of 50 µg/L OCP mixture was used for precison tests, twelve independent solutions being analyzed. The obtained values for the repeatability standard deviation (sr) ranged from 1.30 µg/L to 2.44 µg/L, while the values for the relative standard deviation (RSDr) were between 2.58% and 4.79%.

3.4. Sensitivity

For the sensitivity tests (detection and qauntification limits) a solution of 2 µg/L OCP mixture was used, five sub-samples were analyzed. The detection limits varied in the range of 0.37 µg/L ÷ 0.52 µg/L while the quantification limits varied in the range of 1.24 µg/L ÷ 1.73 µg/L.

3.5. Tests on real samples

3.5.1. Sample preparation

To verify the performance parameters of the method on environmental sapmles, four wastewater samples and four soil samples without any OCPs were selected. To these samples, a standard pesticide mixture solution was added. The extraction from wastewater samples was done using hexane as non-polar solvent in a separatory funnel. For soil samples the extraction was carried out in 50 mL centrifuge tubes using acetone as polar solvent and hexane as non-polar solvent.

3.5.2. Precision

The tests were conducted identically as earlier by contaminating the samples with OCP mix solution obtaining a 50 µg/L concentration. The repeatability of the GC-µECD was characterized by values of the standard deviations (RSDr) which varied in the range of 5.6 % ÷ 10.2% for wastewater and 5.7 % ÷ 10.1% for soil.

To confirm the precision, the method was applied to a total of three replicated tests per matrix, performed by two analysts on four different days. For each set of determinations, the average concentration, standard deviation value, and relative standard deviation (RSD) were calculated. The experiments to determine intermediate precision were conducted using OCP-free samples contaminated with a concentration of 50 µg/L.

The intermediate precision of the method (RSDR) presented values in the range 9.19% to 12.03% for wastewater and 9.12% to 11.96% for soil. These values were situated under 15%, a recommended value for the tested concentrations, therefore the method can be considered precise.

3.5.3. Sensitivity and recovery

The LOQ limits were tested by contaminating the samples with OCP mix solution obtaining a 2 μg/L concentration. The quantification limits varied in the range of 2 μg/L ÷ 3.2 μg/L for wastewater samples and 2 mg/kg ÷ 2.6 mg/kg for soil samples. The recovery percentages after applying the extraction method on the soil samples were situated in the range of 91 % to 110%.

4. CONCLUSIONS

Organochlorine pesticides are synthetic chemical compounds with high stability, leading to their persistence in the environment for extended periods. Their harmful effects have been reported as a result of studies conducted on flora, fauna, and human beings. Gas chromatography with electron capture detection is considered a suitable method for the quantitative determination of organochlorine pesticides. The determination of the content of organochlorine pesticides in environmental samples was performed on a new GC-ECD 8890B Agilent equipment, following, following the in force international standards. From the laboratory verification of the performance parameters of the methods for determining organochlorine pesticides in wastewater and soil, it was observed that the methods are linear, precise, and have suitable detection and quantification limits for the intended purpose. Analysis through GC-MS technique was also conducted to confirm the structural identification of the compounds and their elution order from the column.

5. Acknowledgements

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