



Research article

Techniques for quantification of organochlorine pesticides from a validated method by using gas chromatography-electron capture detector

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ABSTRACT

Organochlorine pesticides (OCPs) are persistent organic compounds found in aquatic environments worldwide. A well-validated and well-established analytical method is crucial for detecting OCPs in the environment. In this study, an analytical method for quantifying OCPs in water was developed and evaluated. Here, the range of linearity, reproducibility, uncertainty, specificity, method detection limits (MDL), and special emphasis on detection and quantitation limits were assessed. Recovery studies were performed to measure the accuracy and precision of the method. This method exhibited excellent linearity in the range of 2.5–20 µg/L for all compounds. As none of the targeted compounds was detected in the chromatograms of the blank sample with no baseline noise, the limits of detection (LOD) and limits of quantification (LOQ) were determined using the linear regression method, external calibration curve slope, and laboratory fortified blank-based detection. All compounds showed different LOD and LOQ values, depending on the approach used. In particular, endosulfan sulfate, methoxychlor, endrin ketone, H. epoxide, heptachlor, and 4,4'-DDT exhibited high detection limits. The recovery percentage of the 15 compounds at 5 µg/L spiked concentration was between 50 and 150 %, which is consistent with the accuracy of the APHA method. Except for endosulfan sulfate, the relative standard deviations of all other compounds were below 20 %, indicating good precision. This method has also been applied to real water samples. This validation technique is reliable, sensitive, simple, rapid, easy to comprehend, and reproducible. The application of this method in the real water samples was also conducted. Only α -BHC and γ -Chlordane were detected in the water sample.

1. Introduction

Organochlorine pesticides are a class of pesticides used extensively worldwide from the 1950s to the 1980s to protect cultivated plants from pests [1,2]. In the Stockholm Convention, 2001, firstly, nine OCPs namely aldrin, chlordane, dichlorodiphenyltrichloroethanes (DDTs), dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, and toxaphene, and later four OCPs (α -, β -,

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γ -hexachlorocyclohexane, chlordecone) and pentachlorobenzene, an impurity found in pesticides have been annexed for their persistent, mutagenic, carcinogenic, bio-accumulative properties, etc. These ubiquitous pollutants are lipophilic, chemically stable, and non-biodegradable in nature; therefore, they persist in the environment for a long time and reach the food chain [2]. Previous studies have reported the availability of OCPs in different environmental matrices such as air, water, soil, sediment, flora, and fauna worldwide, although the application of these chemicals has been banned in many developed and developing countries.

Bangladesh has banned all applications of OCPs under the Environmental Conservation Act of 1995 [3,4]. However, these compounds are still being used in agricultural products [5], preservation, and extended storage of dried fish [6]. As a part of the Stockholm Convention, the production and application of DDT have also been banned, although DDT along with other OCP residues is reported to be found in freshwater, seawater, soil, sediment, milk, fish, eggs, cattle, and poultry feed [4,7]. Hence, it is crucial to determine the exact levels of OCP residues in different matrices to confirm whether the available level is harmful to the ecosystem. A validated and optimized method for OCPs will provide more opportunities to detect such high transportation potential compounds in different environmental compartments, various foodstuffs, animal feed, human and animal bodies, etc.

For decades, a high-resolution gas chromatograph with an electron capture detection (ECD) system has been used as a reference method to evaluate trace levels of OCPs in a variety of environmental matrices. The high sensitivity to halogenated compounds with low cost and readiness makes GC-ECD a successful and suitable analytical approach for environmental pollutants, such as PCBs and OCPs [19]. The main advantages of gas chromatography are its high selectivity and sensitivity for semi-volatile and volatile organic chemicals as well as its accuracy, precision, and resolution over a wide range of concentrations. As a result, GC-ECD has been reported to be highly sensitive and is widely used for semi-volatile OCP determination [1,20].

The validation of analytical methods is a prerequisite for any analytical study. Changes in the development of analytical methods cause variations in data quality. Detecting and quantifying organic chemicals in environmental samples is a difficult task and many researchers adopted different techniques before sample analysis [8–14] to produce reliable data. Also, various factors such as analytical sensitivity and selectivity, method validation and quality control, matrix effects in instrumental analysis, and analytical method reproducibility [15–18] are considered prior to data generation. In addition, analyzing the multiple OCP residues from a standard mixture while optimizing the method validation is a challenge for new researchers. The published research journals for analyzing organic pollutants such as OCPs briefly described the methodology of the analytic procedures, causing confusion and questions among the researchers who are eager to execute similar research. This study proposes the simplest reproducible validated method with rigorous steps of establishing the limit of detection (LOD), limit of quantification (LOQ), method detection limit (MDL), and achieving accuracy, and precision.

This work presents an accurate and practical analytically validated approach for the routine identification of multiple OCP residues in water matrices using a gas chromatography-electron capture detector (GC-ECD). Special emphasis was placed on the experimental methods and calculations of LOD and LOQ for 20 OCPs applying three different methods (an external calibration method, linear regression method, and laboratory-fortified blank-based method). The objectives of this study are to develop and validate a reproducible analytical method for the quantification of 20 organochlorine pesticides residue (α -BHC, β -BHC, γ -BHC, δ -BHC, heptachlor, aldrin, heptachlor epoxide, γ -chlordane, α -chlordane, α -endosulfan, 4,4'-DDE, dieldrin, endrin, 4,4'-DDD, β -endosulfan, 4,4'-DDT, endrin aldehyde, endosulfan sulfate, methoxychlor, and endrin ketone) and to evaluate the method for OCPs residue assessment in water in terms of specificity, precision, linearity, recoveries, and limits of detection and quantification. Also, this technique was applied to environmental samples to confirm its efficacy in real-life applications.

Table 1
Chromatogram properties of GC-ECD for 5 $\mu\text{g/L}$ standard solution.

Name of analyte	Retention time (min)	Area	Height	Concentration
α -BHC	14.85	4355	1103	5.000
γ -BHC	15.97	2369	465	5.000
β -BHC	16.22	2726	661	5.000
δ -BHC	16.91	2949	700	5.000
Heptachlor	17.69	1371	326	5.00
Aldrin	18.81	5439	1226	4.502
H. epoxide	20.80	444	82	2.266
γ -Chlordane	20.99	5648	1221	4.823
α -Chlordane	21.44	5732	1258	5.000
α -endosulfan	21.92	6020	1264	5.000
4,4'-DDE	22.01	5624	1218	5.000
Dieldrin	22.49	5450	1111	4.272
Endrin	23.36	5427	1052	5.000
4,4'-DDD	24.24	8312	1429	5.000
β -endosulfan	25.09	4064	829	5.000
Endrin aldehyde	25.43	2211	440	4.027
4,4'-DDT	26.65	5035	967	3.986
Endosulfan sulfate	27.63	675	111	5.000
Methoxychlor	28.34	1898	351	5.000
Endrin ketone	30.06	1332	216	5.000

2. Materials and methods

2.1. Chemicals

The organochlorine pesticides investigated in this study are listed in Table 1. EPA CLP Organochlorine pesticide standard of approximately 2000 µg/L (approx. 99 %) in toluene:hexane (1:1) was purchased from Sigma-Aldrich for this study. Analytical graded anhydrous sodium sulfate (99 %), n-hexane (95 %), and acetone (99.8 %) were used for the sample and were obtained from Sigma-Aldrich. Deionized water was generated from a DI water plant (MAR-100. Mafco).

2.2. Range of linearity

The linearity is known as the ability of an analytical study to achieve test findings that are in direct proportionate to the concentration (quantity) of analyte in the sample (within a certain range) [21,22]. To assess this, working standards ranging from 1 to 100 µg/L were prepared in n-hexane through series dilution. A minimum of five repetitions of each concentration were injected into the GC. The correlation coefficient (R^2), calibration curve equations, and linearity range were evaluated for each OCP.

2.3. Method detection limit

The minimum concentration of OCPs that can be reported with 99 % confidence that the measured concentration of the analyte is greater than zero and separable from blank results of the method is known as the detection limit for OCPs. In this study, MDLs were assessed from spike concentrations and blanks prepared in deionized water and analyzed. The MDLs were calculated by multiplying the standard deviation (SD) with the student's t-value, as shown in Eq. (1) at ($n-1 = 5$) degrees of freedom at the 99 % confidence limit (LCP, 1996) [11].

$$\text{MDL} = \text{SD} \times \text{t-value} \quad (1)$$

2.4. Recovery study

For the recovery experiments, organic-free deionized water was spiked with 5 µg/L stock standard solutions. Briefly, 250 mL of organic-free deionized water was placed in a 500 mL separatory funnel. Then, a required amount of acetone and n-hexane (1:1) was added to the measured sample, shaken vigorously for 30 min, and allowed to settle for 30 min. After settling, the supernatant was separated and collected in a round-bottom flask. This process was repeated twice. Anhydrous Na_2SO_4 was added to the final combined extracts to make them moisture-free and then concentrated to 2 mL using a rotary evaporator. Finally, the sample was prepared for GC-ECD injection. Four spiking samples were prepared and analyzed on the same day (intraday). The accuracy and precision were calculated from the recovery percentage and relative standard deviation, respectively.

2.5. Limit of detection (LOD) and limit of quantification (LOQ)

Generally, signal-to-noise ratios (S/N) of three and ten are accepted for the LOD and LOQ estimation, respectively. This method is commonly applied to analytical methods that exhibit baseline noise [23]. As no baseline noise was observed in any data, the signal-to-noise ratio was not taken into consideration for the LOD and LOQ measurements. In addition, the injected blank samples did not give any peaks in the chromatograms; as a result, the blank basis standard deviation did not account for the LOD and LOQ determination in this study. In this context, the slope of the calibration curve is a suitable procedure in analytical chemistry for determining the limit of detection and quantification of each compound. An external calibration method was used in this study. The detection and quantification limits were calculated based on slope. They were obtained by plotting the responses of the analytes against a series of standard mixture concentration curves. The LOD and LOQ were calculated using Equations (2) and (3) [24], respectively:

$$\text{LOD} = a + \frac{3.3 \times \text{SD}}{m} \quad (2)$$

$$\text{LOQ} = a + \frac{10 \times \text{SD}}{m} \quad (3)$$

where a is the y-intercept, SD is the standard deviation of the analyte response, and m is the slope of the calibration curve.

Another method to detect LOD and LOQ in this analytical procedure was evaluated using linear regression. The LOD and LOQ were obtained by plotting the area vs. concentration curves of the corresponding calibration standards. The standard deviation of the response (SD) and slope of the curve (m) were then calculated. equations (4) and (5) for the LOD and LOQ measurements are given below

$$\text{LOD} = \frac{3.3 \times \text{SD}}{m} \quad (4)$$

$$\text{LOD} = \frac{3.3 \times \text{SD}}{m} \quad (5)$$

In addition, a laboratory-fortified blank-based detection limit was applied for spiked 2.5 µg/L with five replicates. It displayed another range of detection limits for 20 organochlorine pesticides. In addition, the method detection limits were assessed for these two spiked concentrations based on their standard deviations and t-values.

2.6. GC-ECD condition

Twenty OCPs were analyzed at a series of diluted working standard concentrations using a gas chromatograph (Shimadzu 2010 plus) equipped with a 63 Ni electron capture detector (ECD). A range of working standards of 1 µL volume was injected at 200 °C in split mode (split ratio: 10.0), where the pressure was 31.3 kPa and the carrier gas flow rate was 10 mL/min. A tx-CLPesticides (fused silica) proprietary Crossbond phasecolumn of 30 m × 0.32 mm ID × 0.50 µm was employed. It was set at 120 °C. Column oven program: Detector temp. 300 °C, initial temperature 120 °C held for 1 min, ramp temperature: 15 °C/min to 200 °C at hold time 1 min, then 5 °C/min to 300 °C, and held for 10 min. The total program time was 37.33 min and the ECD gas was nitrogen. All the compounds were distinctively separated, detected, and quantified at a low concentration of 2.5 µg/L. However, at 1 µg/L concentration, only 15 compounds were detected and quantified at respective retention times (Fig. 1). The chromatogram properties at 5 µg/L spike concentration are given in Table 1.

Through ERA's (Environmental Resource Associates) proficiency testing programs (PT) and certified reference material (CRM), this method was validated for the analysis of all 20 OCPs in GC-ECD to acquire ISO 17025:2017. For GC-ECD detector sensitivity and system repeatability test check, gamma-BHC/n-hexane solution with a concentration of 0.1 ng/µL was used as the control standard.

3. Result and discussion

3.1. OCPs

Twenty organochlorine compounds such as α-BHC, γ-BHC, β-BHC, δ-BHC, heptachlor, aldrin, H. epoxide, γ-chlordane, α-chlordane, α-endosulfan, 4,4'-DDE, dieldrin, endrin, 4,4'-DDD, β-endosulfan, endrin aldehyde, 4,4'-DDT, endosulfan sulfate, methoxychlor, and endrin ketone were considered for the study.

3.2. Specificity

The ability to precisely check an assay even in the presence of other components (such as contaminants, disruptive products, and matrix components) is known as the specificity of the validation technique [25]. Method specificity was assessed by analyzing organic free water de-ionized as a reagent blank sample. No interfering peaks of any of the OCPs, as shown in Fig. 2, were observed in the chromatograms of the blank sample, which would extrapolate positive results.

The chromatogram indicates effective separation of the analytes, with a satisfactory analysis duration and high sensitivity. This demonstrates that the chromatographic conditions are ideal for the separation and simultaneous determination of OCPs.

3.3. Linearity and range

A calibration curve was drawn for each pesticide sample from the response of six standard concentrations (1, 2.5, 5, 10, 20, 40, 50,

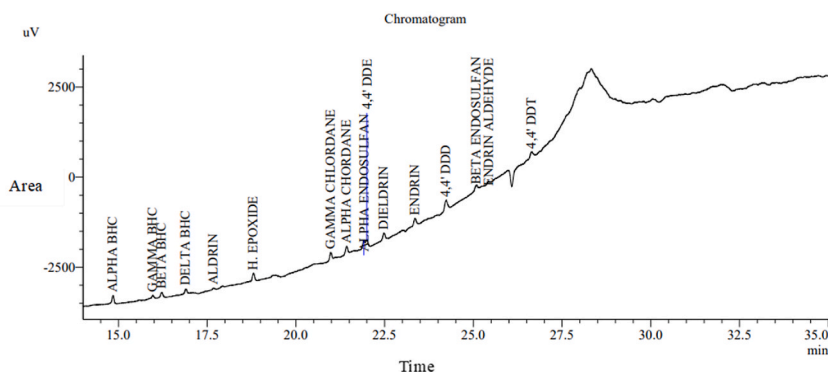


Fig. 1. Chromatogram of 1 µg/L standard solution.

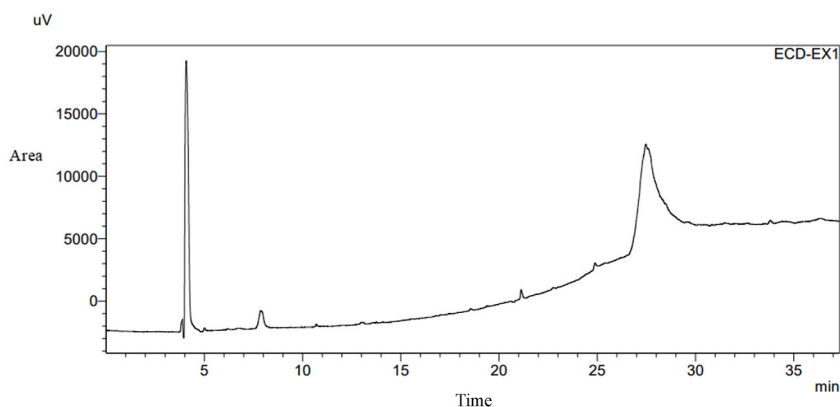


Fig. 2. Chromatogram of the blank sample (n-Hexane).

and 100 µg/L) to determine the linearity of the study. Here, the independent variable (analyte concentration) on the X-axis was plotted against the dependent variable (response from the chromatogram) which was set on the Y-axis to determine the range of linearity for the measurements.

Among all concentrations, only a range of 2.5–20 µg/L exhibits good linearity. As a result, the selected range of linearity of all pesticides ranged from 2.5 to 20 µg/L, as the R^2 values of all pesticides were approximately 1.0, as shown in Fig. 3 along with calibration curves for individual pesticides. This enabled the quantification of these compounds using the external standardization method. The calibration curve intercept and slope of the calibration equations, relative standard deviations (RSDs), and linearity range for each pesticide are listed in Table 2. The correlation coefficient (R^2) of all pesticides ranges from 0.975 to 1 which is coherent with other studies reported in Table S1.

3.4. Limit of detection and limit of quantification

The LOD and LOQ were calculated using three different methods. In the first approach, the required values were obtained from the Origin Lab (Origin Pro 2022), implying linear fit analysis for the methods of linear regression and external calibration. The second and third approaches used the values obtained from the ANOVA table and regression statistics in Excel only for the linear regression method. For each regression analysis, the LOD and LOQ were measured in two ways. The first one is the residual standard deviation of the regression line and the second one is the standard deviation of the Y-intercept of the regression line of the calibration curves.

3.4.1. Linear regression method

The LOD and LOQ for all 20 OCPs were calculated individually within a range of 2.5–20 µg/L and are tabulated in Table 2. The limit of detection and quantification for each pesticide varied from 2.5 to 20 µg/L. The lowest LOD and LOQ were 0.093 µg/L and 0.283 µg/L, respectively. In contrast, γ -BHC exhibited the highest LOD and LOQ values of 1.37 and 4.15 µg/L, respectively. Overall, all 20 pesticides showed good linearity, with fine LOD and LOQ in this range. These values are lower than the studies reported by Fatoki and Awofolu [26] (5.5–20.6 ng/L in water by applying a linear calibration equation). A rising trend in detection limit values appeared in the given order: α -endosulfan < α -BHC < endrin aldehyde < α -chlordane < dieldrin < δ -BHC < γ -chlordane < aldrin < 4,4'-DDD < 4,4'-DDE < endrin < β -BHC < 4,4'-DDT < heptachlor < endosulfan sulfate < β -endosulfan < endrin ketone < methoxychlor < H. epoxide < γ -BHC.

In another LOD and LOQ technique, regression analysis was performed for the different types of datasets. In the first dataset, regression analysis was performed for each compound from repetitive spike concentrations and their respective areas within the range of linearity. The LOD and LOQ were calculated and compared with other studies, as shown in Table 3 and Table S1, respectively. There is a significant difference ($P = 0.013$; $P < 0.05$) between the LODs of the two calculation methods. The ascending order of the compounds is: α -endosulfan < γ -chlordane < aldrin < α -chlordane < dieldrin < β -endosulfan < endrin < 4,4'-DDD < 4,4'-DDE < α -BHC < β -BHC < δ -BHC < endrin aldehyde < γ -BHC < methoxychlor < 4,4'-DDT < H. epoxide < heptachlor < endosulfan sulfate < endrin ketone.

Another regression analysis could be performed while considering the average areas for each concentration in the linear range. Both calibration curve equations were considered for the determination of the LOD and LOQ, as shown in Table 4. A significant difference ($P = 0.033$; $P < 0.05$) has been observed between the LOD groups. The detection limits showed different rising trends and they are: α -endosulfan < dieldrin < α -chlordane < γ -chlordane < aldrin < α -BHC < endrin aldehyde < endrin < β -endosulfan < 4,4'-DDD < 4,4'-DDE < δ -BHC < heptachlor < β -BHC < 4,4'-DDT < γ -BHC < endosulfan sulfate < H. epoxide < endrin ketone < methoxychlor.

3.4.2. External calibration curve slope

The LOD and LOQ values obtained using the slope of the external calibration curve are listed in Table 2. Only α -BHC and endrin

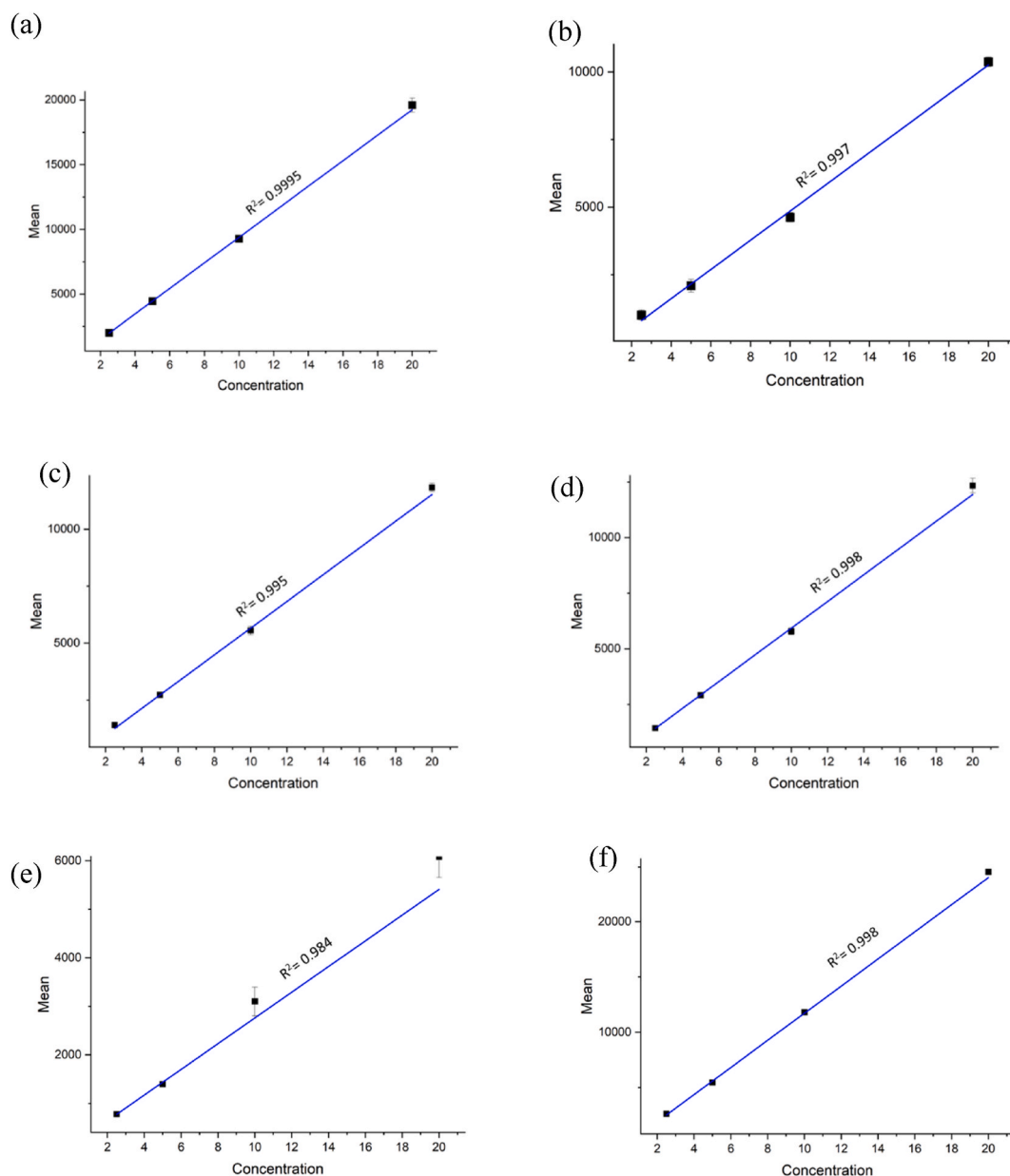


Fig. 3. Calibration curves with correlation of coefficients of all studied OCPs (a) α -BHC, (b) γ -BHC, (c) β -BHC, (d) δ -BHC, (e) Heptachlor, (f) Aldrin, (g) H. epoxide, (h) γ -Chlordane, (i) α -Chlordane, (j) α -endosulfan, (k) 4,4'-DDE, (l) Dieldrin, (m) Endrin, (n) 4,4'-DDD, (o) β -endosulfan, (p) Endrin aldehyde, (q) 4,4'-DDT, (r) Endosulfan sulfate, (s) Methoxychlor, and (t) Endrin ketone.

aldehyde showed negative values as the intercept values of the respective calibration curves were highly negative. Among the remaining compounds, α -chlordane was estimated to have the lowest detection limit (0.044 $\mu\text{g/L}$), and heptachlor was estimated to have the highest detection limit (1.70 $\mu\text{g/L}$). These limits are lower than those reported by Saadati et al. [24] (0.295–2.11 $\mu\text{g/L}$ in water following external calibration). This method of detection and quantitation limits exhibit a different ascending order than linear regression methods. Here the order is: α -chlordane < γ -chlordane < aldrin < H. epoxide < dieldrin < δ -BHC < 4,4'-DDE < β -BHC < methoxychlor < endrin ketone < 4,4'-DDD < endrin < endosulfan sulfate < γ -BHC < β -endosulfan < 4,4'-DDT < heptachlor.

3.4.3. Laboratory fortified blank based detection limit and MDL

The detection limit for laboratory-fortified blank-based detection limit was calculated and is shown in Table 5. The first two low concentrations of the selected range were used in this method. Here, the lowest detection limits for the 2.5 $\mu\text{g/L}$ spike solution were between 0.478 $\mu\text{g/L}$ (α -BHC) and 4.09 $\mu\text{g/L}$ (endosulfan sulfate), with a mean value of $0.922 \pm 1.12 \mu\text{g/L}$, and the quantification limits

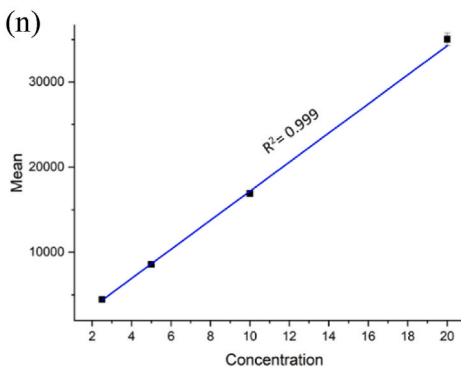
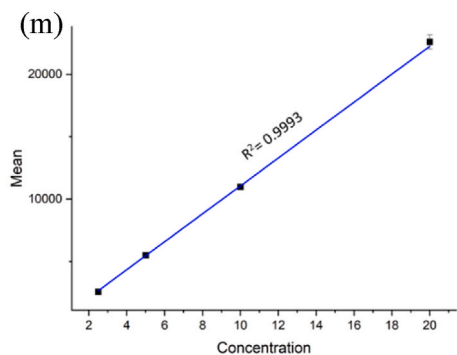
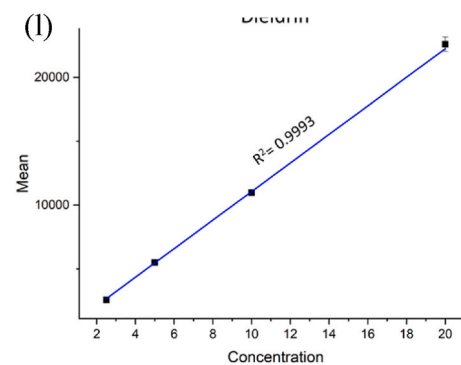
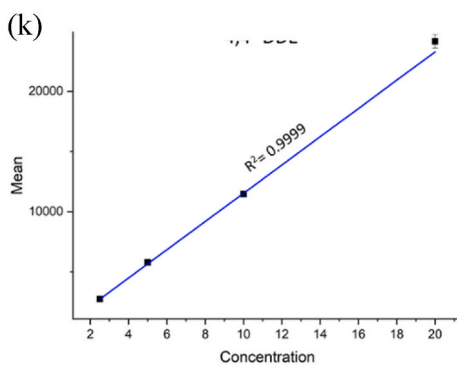
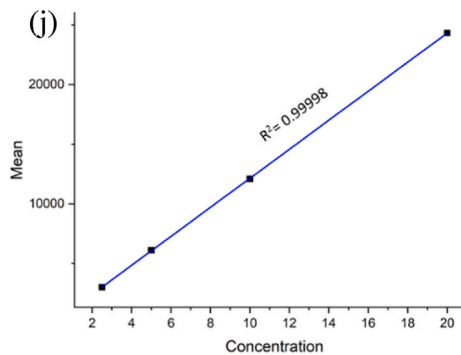
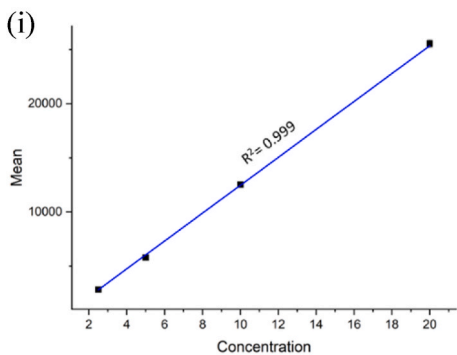
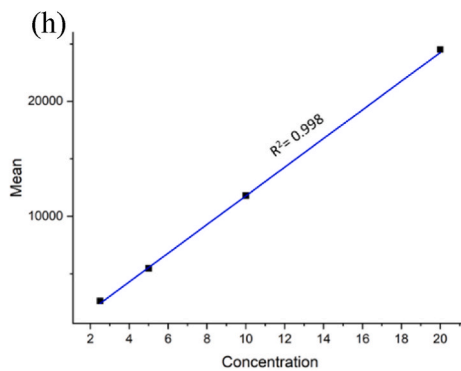
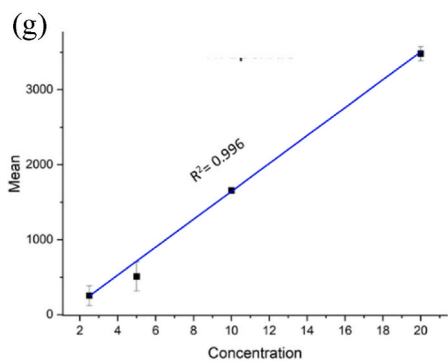


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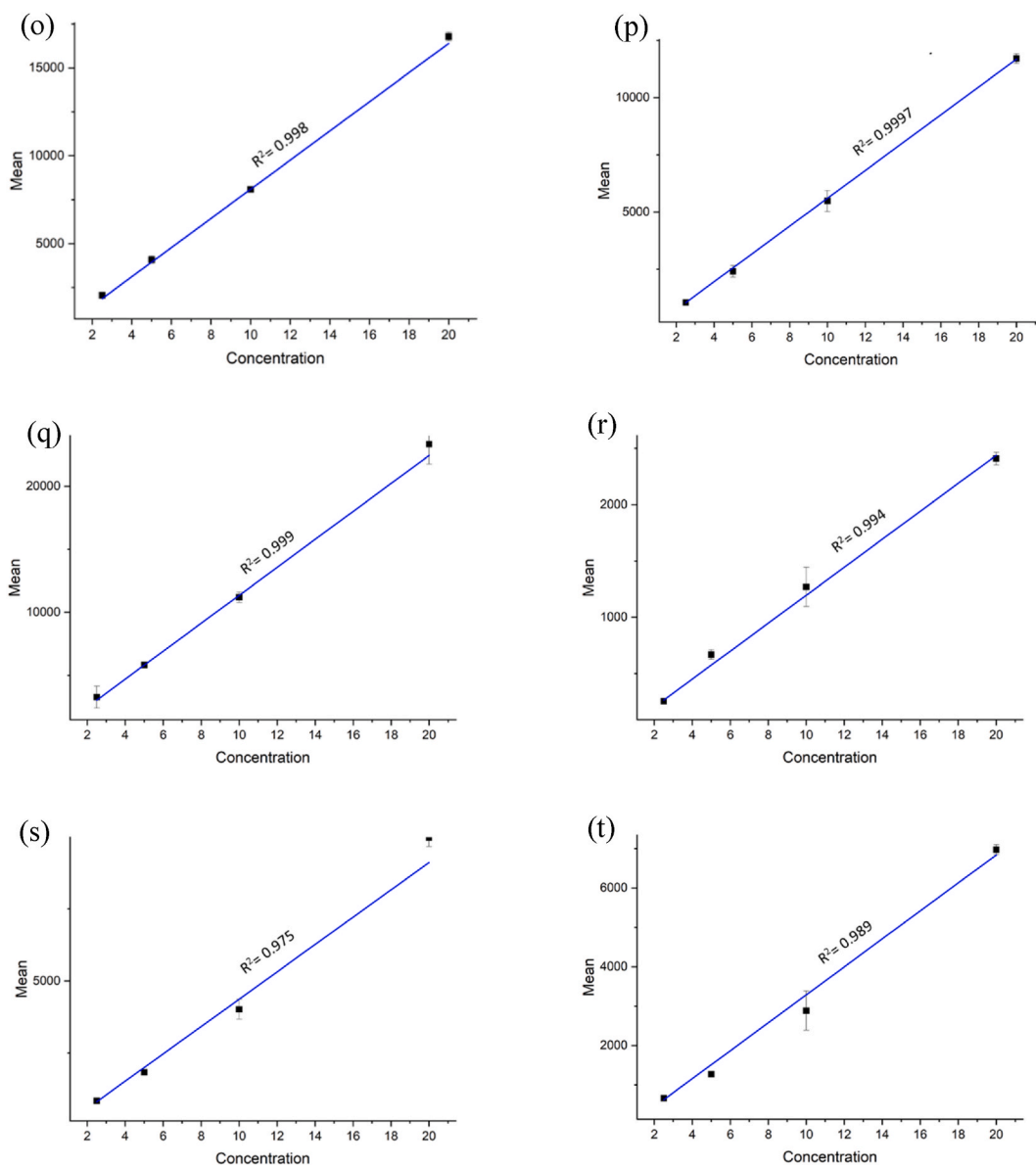


Fig. 3. (continued).

were between 0.319 $\mu\text{g/L}$ (β -endosulfan) and 12.4 $\mu\text{g/L}$ (endosulfan sulfate) with a mean value of $2.79 \pm 3.39 \mu\text{g/L}$. At this concentration, the measured detection limits ranged from 0.100 to 3.89 $\mu\text{g/L}$. The increasing trend of the limits is in the following order: β -endosulfan < 4,4'-DDE < β -BHC < 4,4'-DDD < dieldrin < δ -BHC < aldrin < α -chlordane < α -BHC < γ -chlordane < heptachlor < methoxychlor < endrin aldehyde < γ -BHC < endrin < endrin ketone < H. epoxide < 4,4'-DDT < endosulfan sulfate.

However, the detection and quantification values of the compounds were quite different for the 5 $\mu\text{g/L}$ spiked solutions. The detection limits range from 0.580 to 9.49 $\mu\text{g/L}$ with a mean value of $1.75 \pm 1.30 \mu\text{g/L}$ and the quantification limits range from 0.580 to 17.9 $\mu\text{g/L}$ having a mean value of $5.25 \pm 4.34 \mu\text{g/L}$. The assessed range is higher than those reported by Saadati et al. [24]; Darko and Akoto [27] and Tan and He [28], and closer to the reported detection values of Samoh and Ibrahim [29]. The increasing trend of the limits is in the order of: α -BHC < heptachlor < α -chlordane < aldrin < δ -BHC < β -BHC < endrin < dieldrin < γ -BHC < 4,4'-DDE < γ -chlordane < H. epoxide < 4,4'-DDD < endrin aldehyde < 4,4'-DDT < β -endosulfan < methoxychlor < endrin ketone < endosulfan sulfate.

3.4.4. Accuracy, precision and uncertainty

The precision of the study was evaluated by the repeatability of the recovery employing the relative standard deviation (RSD), and the accuracy was identified by the percentage recovery (%Recovery) on the same day. The recovery criteria stated by the American

Table 2

LOD, LOQ, and calibration curve for linear regression and external calibration methods for 2.5–20 µg/L range.

OCPs	Linear Regression		External Calibration method	
	LOD	LOQ	LOD	LOQ
α -BHC	0.178	0.539	−0.269	0.485
γ-BHC	1.37	4.15	1.13	6.94
β -BHC	0.663	2.01	0.827	3.64
δ -BHC	0.349	1.06	0.492	1.97
Heptachlor	0.740	2.24	1.70	4.84
Aldrin	0.455	1.38	0.317	2.25
H. epoxide	1.36	4.12	0.336	6.11
γ-Chlordane	0.443	1.34	0.137	2.02
α -Chlordane	0.227	0.689	0.045	1.01
α -endosulfan	0.093	0.283	0.153	0.549
4,4'-DDE	0.528	1.60	0.765	3.00
Dieldrin	0.306	0.927	0.422	1.72
Endrin	0.598	1.81	1.00	3.54
4,4'-DDD	0.503	1.53	0.963	3.10
β -endosulfan	0.922	2.79	1.44	5.35
Endrin aldehyde	0.191	0.579	−0.792	0.019
4,4'-DDT	0.688	2.08	1.45	4.36
Endosulfan sulfate	0.833	2.52	1.06	4.59
Methoxychlor	1.08	3.28	0.855	5.45
Endrin ketone	1.00	3.04	0.865	5.12

Table 3

LOD and LOQ measurement from regression line obtained from repetitive spike concentrations for a range of 2.5–20 µg/L.

OCPs	Residual SD of the regression line		SD of Y-intercept of the regression line	
	LOD	LOQ	LOD	LOQ
α -BHC	0.962	2.91	1.65	5.01
γ-BHC	1.55	4.68	2.66	8.05
β -BHC	1.04	3.14	1.78	5.40
δ -BHC	1.16	3.51	1.99	6.04
Heptachlor	2.64	8.00	4.54	13.8
Aldrin	0.483	1.46	0.831	2.52
H. epoxide	2.57	7.79	4.42	13.4
γ-Chlordane	0.464	1.41	0.798	2.42
α -Chlordane	0.530	1.61	0.912	2.76
α -endosulfan	0.433	1.31	0.744	2.26
4,4'-DDE	0.923	2.80	1.59	4.81
Dieldrin	0.817	2.47	1.40	4.26
Endrin	0.846	2.57	1.46	4.41
4,4'-DDD	0.909	2.76	1.56	4.74
β -endosulfan	0.824	2.50	1.42	4.29
Endrin aldehyde	1.43	4.33	2.46	7.44
4,4'-DDT	2.54	7.70	4.37	13.2
Endosulfan sulfate	2.70	8.17	4.64	14.1
Methoxychlor	2.45	7.44	4.22	12.8
Endrin ketone	2.89	8.77	4.97	15.1

Public Health Association (APHA 2012) range from 50 to 150 %, with values of ≤ 20 % relative standard deviation (%RSD). The intra-day precision was calculated from eight replicates as the relative standard deviation (%RSD) of the results was executed on the same day. From [Table 6](#), the recovery of all compounds is within the given range, except for H. epoxide, 4,4'-DDE, 4,4'-DDD, endosulfan sulfate, and methoxychlor. The other 15 OCPs showed good accuracy. The %RSD of the recovery study for 5 µg/L in de-ionized water was between 3.24 % and 22.7 %. Only endosulfan sulfate was above the reference value, while the other 19 OCPs showed good precision.

3.5. Application in environmental water sample

Real sample analyses are usually performed after method validation to evaluate the applicability of the method. This technique was used to assess the 20 OCPs in an urban river in Bangladesh, and the water samples were collected from six sampling sites ([Supplementary Fig. S1](#)) while maintaining the proper sampling procedure. The study area is located adjacent to an industrial area. Three water samples from each sampling site have been collected during the low tide in post-monsoon season. All samples were subjected to LLE extraction as instructed in [section 2.4](#) before analysis using the standard GC-ECD technique. To test the carry-over effect, blank

Table 4

LOD and LOQ measurement from regression line obtained from concentration vs average area for a range of 2.5–20 µg/L.

OCPs	Residual SD of the regression line		SD of Y-intercept of the regression line	
	LOD	LOQ	LOD	LOQ
α -BHC	0.511	1.55	0.878	2.66
γ -BHC	1.44	4.36	2.47	7.50
β -BHC	1.03	3.12	1.77	5.37
δ -BHC	0.986	2.99	1.70	5.14
Heptachlor	1.01	3.07	1.74	5.27
Aldrin	0.428	1.30	0.736	2.23
H. epoxide	2.11	6.40	3.63	10.1
γ -Chlordane	0.422	1.28	0.726	2.20
α -Chlordane	0.394	1.19	0.677	2.05
α -endosulfan	0.130	0.395	0.224	0.679
4,4'-DDE	0.726	2.20	1.25	3.78
Dieldrin	0.344	1.04	0.592	1.79
Endrin	0.650	1.97	1.12	3.39
4,4'-DDD	0.713	2.16	1.23	3.72
β -endosulfan	0.657	1.99	1.13	3.42
Endrin aldehyde	0.545	1.65	0.937	2.84
4,4'-DDT	1.12	3.38	1.92	5.81
Endosulfan sulfate	1.83	5.54	3.15	9.53
Methoxychlor	2.67	8.10	4.60	13.9
Endrin ketone	2.58	7.82	4.44	13.4

Table 5

Calculation of LOD and LOQ based on laboratory fortified blank and their respective method detection limits.

OCPs	MDL for 2.5 µg/L	Laboratory fortified blank based detection limit for 2.5 µg/L (n = 5)			MDL for 5 µg/L	Laboratory fortified blank based detection limit for 5 µg/L (n = 5)		
		SD	LOD = SD × 3.3	LOQ = SD × 10		SD	LOD = SD × 3.3	LOQ = SD × 10
		α -BHC	0.455	0.145		0.478	1.45	0.580
γ -BHC	0.874	0.278	0.919	2.78	0.961	0.278	0.919	2.78
β -BHC	0.171	0.054	0.179	0.544	0.807	0.054	0.179	0.544
δ -BHC	0.339	0.108	0.357	1.08	0.735	0.108	0.357	1.08
Heptachlor	0.540	0.172	0.568	1.72	0.593	0.172	0.568	1.72
Aldrin	0.368	0.117	0.387	1.17	0.698	0.117	0.387	1.17
H. epoxide	1.44	0.458	1.51	4.58	1.17	0.458	1.51	4.58
γ -Chlordane	0.526	0.168	0.553	1.68	1.12	0.168	0.553	1.68
α -Chlordane	0.422	0.134	0.443	1.34	0.657	0.134	0.443	1.34
α -endosulfan	0.553	0.176	0.581	1.76	4.74	0.176	0.581	1.76
4,4'-DDE	0.127	0.040	0.133	0.403	0.972	0.040	0.133	0.403
Dieldrin	0.330	0.105	0.347	1.05	0.922	0.105	0.347	1.05
Endrin	0.910	0.290	0.956	2.90	0.846	0.290	0.956	2.90
4,4'-DDD	0.190	0.061	0.200	0.607	1.29	0.061	0.200	0.607
β -endosulfan	0.100	0.032	0.105	0.319	1.83	0.032	0.105	0.319
Endrin aldehyde	0.631	0.201	0.663	2.01	1.32	0.201	0.663	2.01
4,4'-DDT	3.69	1.18	3.87	11.75	1.61	1.18	3.88	11.752
Endosulfan sulfate	3.89	1.24	4.09	12.4	5.38	1.24	4.092	12.4
Methoxychlor	0.569	0.181	0.598	1.81	2.63	0.181	0.598	1.81
Endrin ketone	1.42	0.451	1.49	4.51	2.64	0.451	1.49	4.51

samples were measured prior to the real samples. The integrity of the experimental data has been ensured by running QC samples. In the study, the 5 µg/L OCP standard was used as the control standard. After every 10 samples, 1 control sample has been ran.

All the water samples showed detectable concentrations of OCPs. OCPs are poorly water-soluble and are mainly adsorbed on the total organic carbon and dissolved organic carbon. No pesticides were found in the actual samples at concentrations below the detection limit of the method. Out of 20 OCPs, only α-BHC and γ-Chlordane were present in the water samples, considering the limit of detections. α-BHC (0.024–0.113 µg/L) is only above the detection limit according to the external calibration method. γ-Chlordane (0.286–2.85 µg/L) exceeds the detection limit in all methods.

4. Conclusion

This study elaborately demonstrates how a method validation study can be carried out for 20 OCPs in water using GC-ECD. In the validation steps, key parameters (specificity, linearity, limit of detection, limit of quantification, and recovery study) were assessed, and special emphasis was given to the LOD and LOQ calculation methods. Among the wide range of spike concentrations, only 2.5–20

Table 6

%Recovery and %RSD of the recovery study at 5 µg/L spike in deionized water.

OCPs	% Recovery (n = 8)		%RSD	*Expanded uncertainty
	Min	Max		
α -BHC	101.5	112.4	3.62	0.077
γ -BHC	126.1	146.2	4.70	0.100
β -BHC	118.4	135.5	4.25	0.091
δ -BHC	124.2	137.5	3.76	0.080
Heptachlor	111.0	123.3	3.41	0.073
Aldrin	127.5	139.6	3.48	0.074
H. epoxide	40.8	62.4	15.3	0.326
γ -Chlordane	116.8	141.3	5.66	0.121
α -Chlordane	129.8	142.0	3.24	0.069
α -endosulfan	46.3	148.1	4.41	0.094
4,4'-DDE	165.9	185.1	3.76	0.080
Dieldrin	116.7	137.3	4.81	0.103
Endrin	129.9	147.7	4.09	0.088
4,4'-DDD	134.1	162.9	5.97	0.128
β -endosulfan	76.34	110.7	13.1	0.280
Endrin aldehyde	68.96	100.7	9.99	0.214
4,4'-DDT	105.2	135.7	9.14	0.195
Endosulfan sulfate	116.0	216.9	22.7	0.485
Methoxychlor	126.4	188.1	11.4	0.244
Endrin ketone	76.94	133.1	16.4	0.350

Expanded uncertainty = average %RSD*K; K = 2 (11).

%RSD = standard deviation of concentration/average of concentration.

µg/L showed excellent linearity for all the compounds ($R^2 \sim 1$). As no compound peak was observed in the blank samples, the calibration curve and external calibration curve-based LOD and LOQ were obtained in two software (Origin Lab and MS Excel). The LOD results obtained from the Origin Lab ranged from 1.37 to 0.093 µg/L (linear regression) and from 1.703 to 0.792 µg/L (external calibration). However, the LODs and LOQs determined from the residual standard deviation of the regression line from the regression analyses exhibited smaller values than the calculation based on the standard deviation of the Y-intercept. The method detection limits are varied from 0.100 to 3.89 µg/L and from 0.580 to 9.49 µg/L for 2.5 µg/L and 5 µg/L, respectively. Recovery studies at 5 µg/L in deionized water showed that this method is accurate for 15 compounds and precise for nearly all compounds except endosulfan sulfate.

A similar study using the internal calibration method is encouraged for future studies. Also, the extraction of real or environmental water samples can be affected by the contents of other constituents. The extraction efficiency of OCPs in water in different concentrations of other constituents such as organic matter, dissolved and/or suspended solids, etc. can be analyzed in the future.

Additional information

No additional information is available for this paper.

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During the preparation of this work the author(s) did not use any generative AI or AI assisted technologies.

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CRedit authorship contribution statement

Nushrat Jahan Chowdhury: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Conceptualization. **Md. Ahedul Akbor:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Aynun Nahar:** Writing – review & editing, Data curation. **Md. Aftab Ali Shaikh:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e34548>.

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