

Development, Validation, and Application of a Method Based on Reverse-Phase HPLC for the Simultaneous Determination of Six Organochlorine Pesticides in Surface and Groundwater Samples Collected from Northeast Libya

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Abstract: The contamination by organochlorine pesticides (OCPs) of the selected sites in the eastern region of Libya was investigated to estimate the current status of pollution in surface and groundwater sources. This study was conducted to determine the concentrations of some OCPs residue in surface and groundwater samples collected from thirty different sites around Al-Jabal Al-Akhdar northeast of Libya during the summer, autumn, and winter seasons of 2016. A simple and rapid method based on the liquid-liquid extraction method followed by an HPLC with reversed-phase was developed to determine six OCPs in water samples simultaneously. An HPLC instrument was supplied with a C₁₈ column (250 mm × 4 mm; 4.6 μm particle size) and a UV detector at 238 nm. The mobile phase was composed of 0.1% propanol in water and acetonitrile (25/75, v/v). The method was validated using reference standards of these six OPCs at different concentration levels and shows good linearity in the concentrations between 5.0 and 25 μg mL⁻¹. The LOD and LOQ ranged from 0.71 to 2.24 μg mL⁻¹ and 2.16 to 6.79 μg mL⁻¹, respectively. Relative standard deviation (%RSD) ranged from 0.026 to 0.673 %. The only OCP residue found in the area of study was 2, 4-D, with a variation of its residue levels during the seasons. The overall results showed that surface water was more polluted with 2, 4-D than groundwater, which was detected in the wide range of concentration of 0.037 to 0.385 μg mL⁻¹, 0.003 to 0.047 μg mL⁻¹ and 0.012 to 0.039 μg mL⁻¹ during summer, autumn, and winter, respectively.

Keywords: HPLC; Validation; Residues; Surface Water; Groundwater.

INTRODUCTION

Pesticides play a vital role in agricultural production to prevent or decrease damage by pests. And as a result can enhance the yield and the quality of production even as far as cosmetic demand, which is regularly vital to consumers (Cooper & Dobson, 2007; Oerke & Dehne, 2004). The nutritional value of food and its safety can be enhanced and improved by pesticides (Cooper & Dobson, 2007; Smilanick, 2008). Data from several studies suggest that Organochlorines pesticides (OCPs) are among

the pesticides that are used for agricultural purposes all over the world. Because of their widespread uses, they are detected in various environmental matrices, such as soil, water, and air (Azad et al., 2012; Mariyono, 2008; Sankaramakrishnan et al., 2005). OCPs are characterized as low hydrophilicity and more lipophilicity substances. And therefore, have potential bioaccumulation in the food chain which poses a significant threat to human health and the environment globally (Afful et al., 2010).

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Furthermore, several studies have reported that the persistence of organochlorine pesticides in soil and water of cultivated land has the opportunity to drain into groundwater in different levels of quantity depending on the nature and chemical characterizations of these pesticides (Jamal, 2011). In addition, results of water and sediment samples from Lake Manyas in Turkey showed that these samples were still contaminated by organochlorine pesticides and their residues, despite the bans on their production and usage over a long time (Erkmen et al., 2013).

Furthermore, the determination of persistent pesticides is of particular importance to the environment in the area of toxicology. Thus, various analytical methods such as GC-ECD (Ismail et al., 2014; Saha et al., 2012); (Hashmi & Menon, 2015), GC-MS (El-Saeid et al., 2011), gas chromatography–nitrogen-phosphorus detection (Dores et al., 2008) HPLC (Ara et al., 2014) have been reported, and many of these methods involve a solid-phase extraction and/or liquid-liquid extraction procedures prior to the detection by analytical techniques (El-Osmani et al., 2014; Shamsipur et al., 2016); (Leong et al., 2007); (Shakerkhatibi et al., 2014).

Extensive research has shown that OCPs are commonly analysed and detected using Gas chromatography techniques with different detectors (Tata Rao et al., 2014); (Ize-Iyamu et al., 2007). Recently, Azad *et al.* (2012) analysed six OCPs in four sites at four seasons using a liquid-liquid extraction technique and then gas chromatograph equipped with an electron capture detector (GC-ECD). Also they used Soxhlet extraction for fish and sediment samples followed by clean up and then gas chromatography. They found DDE as a predominant residue in all analysed samples, and the lowest levels of OCPs were related to Heptachlor and Chlordane, which none of them were found in water samples (Azad et al., 2012).

Also, Lari *et al.* (2014) evaluated the OCPs and organophosphates pesticides as a potential pollutants and risks to human health. They utilized liquid-liquid extraction followed by the GC-MS technique for evaluating these pesticides in surface water and groundwater. The surface water was found to be more contaminated than groundwater, with a higher number and concentration of pesticides (Lari et al., 2014a). In the same vein, Jayashree and Vasudevan (2007) analyzed some OCPs, chosen to know the level of their contamination in the groundwater of the Thiruvallur district, Tamil Nadu, India. The samples were found highly contaminated with DDT, HCH, endosulfan, and their derivatives. Their study showed that groundwater samples were highly contaminated with organochlorine residues (Jayashree & Vasudevan, 2007). Recently, researchers have shown an increased interest in using GC equipped with ECD and a pulsed flame photometric detector to analyze OCPs in water and sediment samples. In some cases, the residues were further confirmed by Gas Chromatograph-Mass Spectrometer-Quadrupole on Electron Ionization (EI) mode (Akoto et al., 2016; Maurya & Kumar, 2013); (Hellar-Kihampa, 2011).

Surveys, such as that conducted by El Bouraie *et al.* (2011) have shown that the contamination of organochlorine pesticides (OCPs) along El Rahway drain, they have determined the concentrations of eighteen OCPs in surface and groundwater samples collected from six different sites during the rainy and dry seasons. The samples were extracted by the liquid-liquid extraction method. Then, determined qualitatively using GC/ECD (Gas chromatograph equipped with an electron capture detector). The commonly found OCP residues included heptachlor, p,p'-DDE, p,p'-DDD, and endrin, and the overall results showed that surface water was more polluted with OCPs than groundwater (El Bouraie et al., 2011).

Previous research has suggested that using solid-phase extraction (SPE) coupled with dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry (GC-MS) provides higher extraction efficiency and a larger preconcentration factor for the determination of pesticides residues (Shamsipur et al., 2016). In addition, solid-phase microextraction (SPME) followed by gas chromatography (GC) techniques have been applied for the analysis of chlorinated pesticides in water, with good reproducibility, low detection limits, and wide linear ranges (Boussahel et al., 2002); (Dores et al., 2008). Okoya *et al.* (2013) investigated levels of OCP residues in drinking water sources using liquid /liquid extraction and supercritical fluid extraction (SFE), and this study concluded that most of the water samples contaminated with OCPs associated with agricultural activities in their areas (Okoya et al., 2013). However, using Static liquid-phase microextraction technique for analysis of OCPs from aqueous samples showed to be quick, inexpensive, and used very little solvent with subsequent analysis by gas chromatography–electron-capture detection (Zhao & Lee, 2001).

On the other hand, HPLC with a UV/visible detector was utilized by Aiken *et al.* They found the concentrations of organochlorine and organophosphorus pesticide residues were significantly higher in the sediment samples when compared to water samples (Akan et al., 2014). As well as Ara *et al.* (2014) who conducted a study to analyse pesticides including OCPs which was carried out with the HPLC technique. Pesticides were found in twelve samples, while Organochlorine was absent (Ara et al., 2014).

The objectives of this study involve the development and validation of the HPLC method to determine the residue of six organochlorine pesticides in surface and groundwater and their compatibility with the recognized maximum limits and the absence of

a database on the level of pollution of pesticides in the water sources and then directed consumers to look at the dangers.

MATERIALS AND METHODS

Sampling site, collection, and storage: The region of the study is located between Wadi Derna [1] (E 22.619346° N 32.727701°) to Al-bayarIII [30] (E 20.522419° N 32.257841°) in the northeastern region of Libya. 9 samples of surface water and 21 groundwater samples were collected in summer, autumn, and winter season 2016. The sources [Codes 1-30] are shown in Figure (1), and a device of Global Positioning System (GPS) was used to locate positions during sampling. Samples were collected by sterilized pre-cleaned stainless metal containers and then saved in clean ice-covered dark glass bottles and immediately transferred to the laboratory. The samples were performed according to the recommendations by D.T.E Hunt and A.L. Wilson (APHA, 1995; Hunt & Wilson, 1986). They were properly labeled and kept at -20 °C before analysis (Uddin et al., 2007).

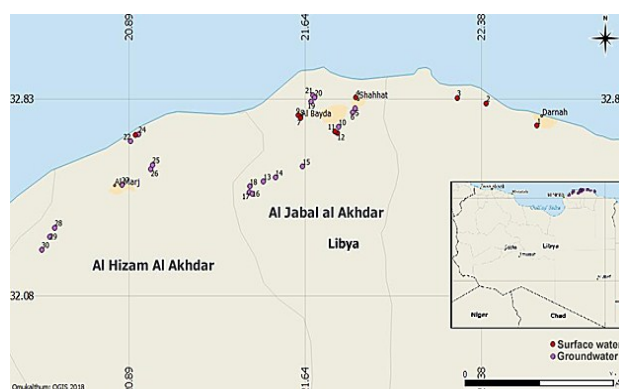


Figure: (1). Study locations that located between Wadi Derna [1] (E 22.619346° N 32.727701°) to Albayar [30] (E 20.522419° N 32.257841°).

Pesticide Standards and chemicals: The individual six organochlorine pesticides (2, 4-D, p,p'-DDE, p,p'-DDT, p,p'-DDD, Dicofol, and Heptachlor) reference standards with a certified purity of 99 % were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC

grade acetonitrile and dichloromethane were obtained from Fisher Scientific (UK). Propan-1-ol was obtained from VWR (UK), water was obtained in-house by Water Distillation Unit. All other reagents and chemicals and solvents used were of analytical grade purchased from Sigma-Aldrich (Germany) included: anhydrous sodium sulfate, acetone, Uracil, and Sodium chloride.

Apparatus and chromatographic conditions

High-performance liquid chromatography was performed with an integrated Spectra system Liquid Chromatograph (Thermo electron corporation, USA) fitted with an in-line degasser, 120-place autoinjector, and single-channel, tunable UV absorbance detector.

Data analysis was carried out using Thermo Electron Corporation software (ChromQuest). The flow rate was 1.0 mL min^{-1} , with an injection volume of 20 mL. Three replicate injections of each calibration standard were performed. The analysis was performed using a reversed-phase stationary phase (ACE C₁₈, 250 mm x 4.6 mm i.d., particle size: 3 μm ; PerkinElmer (Shelton, USA)), and samples were isocratically eluted with a 0.1% Propan-1-ol in water and Acetonitrile (25/75, v/v) at wavelength 238 nm.

Preparation of standard sample for HPLC

Calibration standards: 5 mg of each standard were weighed accurately into a 50 mL volumetric flask and diluted to volume with acetonitrile to give a mixed stock solution. The analytical curve was prepared with a standard in final concentrations of 5-25 $\mu\text{g mL}^{-1}$, originally acquired from a 100 $\mu\text{g mL}^{-1}$ of mixed six OCPs stock solution. All samples were prepared in triplicate.

Samples preparation for HPLC

Extraction of water samples: The method prescribed by the Water Environment Federation (WEF) was used, with some modifications, for the extraction of OCP residues from the water samples (APHA, 1998). A liquid-liquid ex-

traction (LLE) method with dichloromethane was used for extracting OCP residues from the water sample. Water samples were filtered with Whatman No. 1 filter paper to remove debris, and then a 200 mL of filtrate water sample was transferred into a 500 mL glass-separating funnel. Then, 20 g of NaCl was added to produce a salt-out effect. It was thoroughly mixed by inverting the separating funnel three to four times. The sample was extracted thrice with 40 mL dichloromethane (20:10:10), shaken for 3–4 min each time with periodic venting. The combined organic phase was dried by passing it through anhydrous Na₂SO₄ and then in a vacuum rotary evaporator (Heidolph). The dried sample was reconstituted in 1 mL of Acetonitrile, and finally, the aliquot was analyzed by HPLC. These samples were analyzed in triplicate (Lari et al., 2014b).

Analytical method validation

System suitability : A mixture solution of six OCPs with a concentration of 100 $\mu\text{g mL}^{-1}$ was injected three times to evaluate the analytical response. The mean and relative standard deviation (RSD %) were calculated. Theoretical plate number and tailing factor were also observed.

Linearity: The analytical curve was obtained with different concentrations of the mixture standard solution (5– 25 $\mu\text{g mL}^{-1}$). The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis.

Precision: The precision of the assay was determined by repeatability which was evaluated by assaying samples at the same concentration during the same day. Five sample solutions were prepared.

Recovery method (Accuracy): The accuracy was determined by the recovery of known amounts of OCPs standard added to the samples at the beginning of the process. Thus, the recovery experiment was performed by spiked 1 mg of each pesticide standard individually to

200 mL of water and extracted then dried. Then 10 mL of Acetonitrile was added to reach a concentration of $100 \mu\text{g mL}^{-1}$. The percentage recoveries were calculated using the following equation Percentage of recovery = $[(C_E/C_M \times 100)]$, where C_E is the practical concentration obtained from a standard solution of $100 \mu\text{g mL}^{-1}$ of each compound in acetonitrile, and C_M is the spiked concentration after extraction (Chowdhury et al., 2012).

Statistical analysis: Statistical analyses were carried out by analysis of variance (ANOVA) using SPSS 25 software. Differences in pesticides were analyzed by the t-test between the surface and groundwater samples. The statistical significance tests were carried at the 0.05 confidence level ($p < 0.05$). Data between regions and seasons, also interaction was tested using two-way ANOVA and Mean values were analyzed by the Tukey HSD (Honest Significant Difference) test at ($p < 0.05$) levels (Nie et al., 1970).

RESULTS

Optimization of chromatographic conditions

An RP-HPLC method for the simultaneous determination of six OCPs was developed, and excellent separation was only achieved with the C_{18} column length 250 mm.

To optimize the HPLC parameters, isocratic elution with several mobile phase compositions was evaluated to elute all pesticides in the sample in a reasonable amount of time. The separation was increased with more amount of organic solvent in the mobile phase than water. Although run lengths were longer at higher proportions of acetonitrile in the mobile phase, low or no resolution was observed when a higher proportion of water was used. A composition of acetonitrile and water in the mobile phase was varied, and increasing the amount of acetonitrile in the mobile phase was producing the best possible separation within the desired elution time. Flow rates of the mobile phase also varied between 0.5 and 1.0 mL min^{-1} , of which 1.0 mL min^{-1} gave an acceptable resolu-

tion ($R_s > 2$) and better symmetry (< 1.2). Depending on the results of various trials, a mobile phase consisting of acetonitrile: 1% Propan-1-ol in water (75:25 v/v) at ambient temperature was used for this study. Figure 6 shows the separation of all six compounds in reasonable run time, whereby the elution order was 2, 4-D, Dicofol, DDD, Heptachlor, DDT, and DDE.

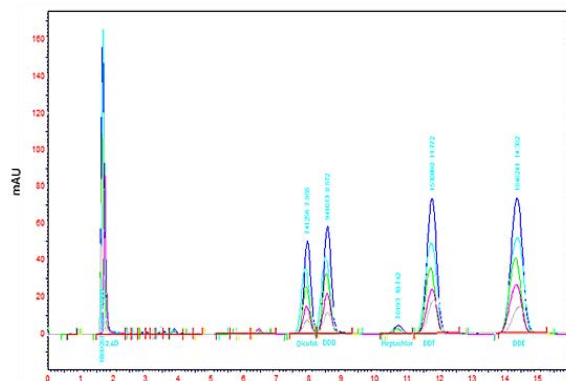


Figure 1: (2). Simultaneous separation of 2, 4-D, Dicofol, DDD, Heptachlor, DDT and DDE. Conditions: Column: C_{18} , mobile phase: acetonitrile: 1% Propan-1-ol in water (75:25 v/v), ambient temperature, flow: 1.0 mL min^{-1} , UV: 238 nm, injection: $20 \mu\text{L}$.

Method validation: The good linearity of the calibration curve was validated by the high value of correlation coefficients of the regression graph. Calibration standards were prepared and demonstrated a linear response ($R^2 = 0.99-0.99$) over a $5-25 \mu\text{g mL}^{-1}$.

The precision of the proposed method was also determined by mixing six OCPs at $5-25 \mu\text{g mL}^{-1}$ of each OCP. And was evaluated in terms of repeatability and expressed as the relative standard deviation (RSD,%). The result of precision ranged from 0.026 to 1.374%, which indicates good repeatability. The recovery of the method can be defined as accuracy. It was calculated of the known concentration level by comparing the measured concentration with spiked concentration as the reported method (Yusiasih et al., 2017) with a number replicate of three. The average result of the recoveries of all pesticides obtained ranged from 84.69 to

98.16%. These values were considered as an acceptable and good result since 70 – 130% recoveries were approved (Demoliner et al., 2010).

The results showed in Table (1) from method validation of 2,4-D, Dicofol, DDD, Heptachlor, DDT, and DDE: Capacity factor (k') ranged from 0.10 (2,4-D) to 8.38 (DDE), Resolution (R) were from 1.13 (Dicofol) to 19.68 (2,4-D), Symmetry factor (A_s) were from 0.95 (Heptachlor) to 1.08 (Dicofol), Selectivity (α) were ranged from 41.6 (2,4-D)

to 1.31 (DDD) and theoretical plate (N) were ranged from 289 (1156) (2,4-D) to 14200.69 (56802.77) (DDE), as well as a slight peak tailing ($A_s \sim 1.2-0.9$) being observed in each case. The limits of detection and quantification were determined to be 1.73 and 5.25 $\mu\text{g mL}^{-1}$ for 2,4-D, 1.73 and 5.26 $\mu\text{g mL}^{-1}$ for Dicofol, 2.24 and 6.79 $\mu\text{g mL}^{-1}$ for DDD, 1.15 and 3.50 $\mu\text{g mL}^{-1}$ for Heptachlor, 0.71, and 2.16 $\mu\text{g mL}^{-1}$ for DDT, 2.04, and 6.16 $\mu\text{g mL}^{-1}$ for DDE, respectively.

Table (1). Summary of validation data for the quantification of OCPs including 2,4-dichlorophenoxyacetic acid, heptachlor, p, p'-DDE, p, p'-DDD, p, p'-DDT and Dicofol in the presence of common adulterants using Brownlee BIO C₁₈ column (250 mm x 4.6 mm id, particle size 5 μm); mobile phase: 0.1% Propan-1-ol in water and Acetonitrile (25:75); detector wavelength: 238 nm.

Names	2,4-D	Dicofol	DDD	Heptachlor	DDT	DDE
t_R (min) ($t_0 = 1.533 \text{ min}^a$)	1.69	7.90	8.55	10.73	11.76	14.38
Capacity factor (k')	0.10	4.16	4.58	6.00	6.67	8.38
Resolution (R)	19.68	1.13	4.03	1.73	5.90	-
Symmetry factor (A_s)	1.05	1.08	1.01	0.95	0.96	1.07
Selectivity (α)	41.6	1.10	1.31	1.11	1.25	-
LOD ^b ($\mu\text{g mL}^{-1}$)	1.73	1.73	2.24	1.15	0.71	2.04
LOQ ^c ($\mu\text{g mL}^{-1}$)	5.25	5.26	6.79	3.50	2.16	6.16

DISCUSSION

In thirty samples collected during each season, 2, 4-D pesticide was found in most seasons and regions. It is noteworthy that p, p'-DDE, p, p'-DDD, p, p'-DDT, Dicofol, and Heptachlor were not detected in all samples that were collected in summer, autumn, and winter season of surface and groundwater. Monitored pesticides in this study were detected with residue values less and greater than MRL's (0.030 $\mu\text{g mL}^{-1}$), according to World Health Organization (WHO) and the Libyan National Center for Standardization and Metrology (LNCMSM) (WHO, 2004) in all collected samples.

However, Data showed that 2,4-D was detected in surface water with concentration ranged from 0.037 $\mu\text{g mL}^{-1}$ (Aldabusia) [3] to 0.385 $\mu\text{g mL}^{-1}$ (Wadi Darna) [1] and detected in groundwater ranged from lower to greater con

centration between 0.013 $\mu\text{g mL}^{-1}$ (Marawa III) [18] to 0.127 $\mu\text{g mL}^{-1}$ (Wasita I) [19]. The histogram shown in Figure 3 indicates that the major residues of 2, 4-D pesticides in surface water were in Wadi Darna [1] (0.38 $\mu\text{g mL}^{-1}$). The five other regions were detected in moderate concentrations, and these were Karsa [2], Aldabusia, Apouloana Shahhat I [4], Saylun Massa III [9] and Tolmeita II [23] with concentration (0.38, 0.063, 0.037, 0.084, 0.061, and 0.048 $\mu\text{g mL}^{-1}$) respectively. Also, the results of this study indicate that three other regions found no present of 2, 4-D in their samples in surface water during the same season.

In groundwater samples, the histogram indicates that 2, 4-D residues were detected in most of the regions, including Shahhat II [4], Shahhat III [5], Massa II [8], Qandula I [13], Qandula II [14], Qandula III [15], Marawa

II[17] , Wasita I[19], Wasita II [20], Tolmeita I[22], Alabyar I[28], and Alabyar III[30] with concentration (0.065, 0.120, 0.037, 0.051, 0.043, 0.034, 0.094, 0.127, 0.090, 0.042, 0.033, and 0.035 $\mu\text{g mL}^{-1}$) respectively which were exceeded MRL (0.030 $\mu\text{g mL}^{-1}$) that determined by World Health Organization (WHO) limits, Libyan National Center for Standardization and Metrology (LNCSM). These findings were in agreement with the previous studies (Adeyemi et al., 2011; Hong et al., 1999; Munn & Gruber, 1997; Que Hee & Sutherland, 1981; Tang et al., 2008) which found the large amount of 2, 4-D as a herbicide. This indicates that 2, 4-D is still used in most countries in the world (Chen et al., 2018). Also, it is still used in neighboring countries such as Egypt, according to Mansour *et al.* study (El-Ghit, 2016; Mansour, 2004).

Histograms shown in Figures (5, 6) indicate 2, 4D residue in the autumn season of water samples collected from nine surface water and twenty-one of groundwater sources in different regions. In the case of surface water samples collected, we found six out of nine samples were contaminated during the autumn season of 2016. 2,4-D was detected with concentrations ranging from 0.003 $\mu\text{g mL}^{-1}$ (Tolmeita II) [23] to 0.047 $\mu\text{g mL}^{-1}$ (Aldabusia) [4] in surface water, which shows that most of the groundwater samples detected had a concentration which ranged between 0.003 $\mu\text{g mL}^{-1}$ (Alabyar III) [30] to 0.036 $\mu\text{g mL}^{-1}$ (Qandula I)[13]. In the autumn season, results from this study showed just 11.11% from surface and groundwater above the (WHO) and (LNCSM), while most samples were lower than the maximum residue limit according to the MRL's level.

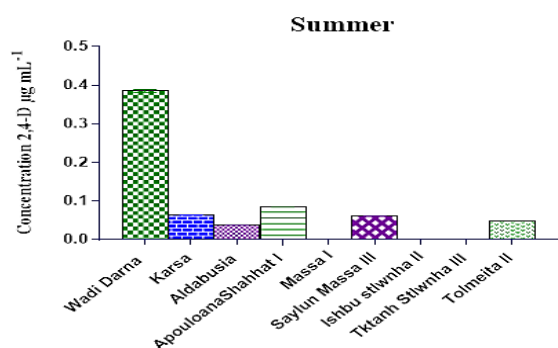


Figure (3). Residues of 2, 4-D pesticides in surface water from different regions of eastern Libya during summer season 2016.

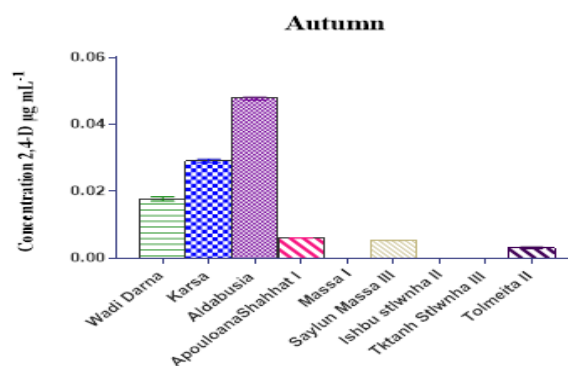


Figure (5). Residues of 2, 4-D pesticides in surface water from different regions of east Libya during the autumn season of 2016.

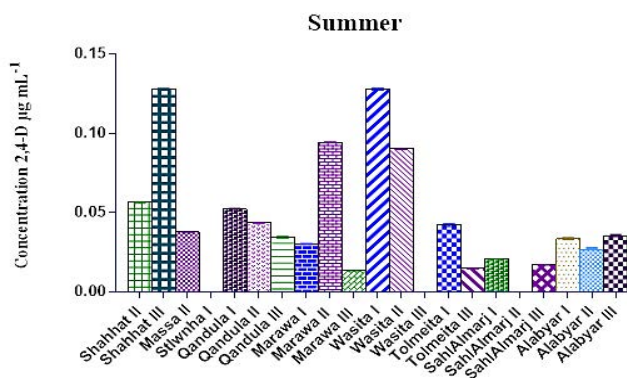


Fig (4). Residues of 2, 4-D pesticides in groundwater from different regions of eastern Libya during summer season 2016.

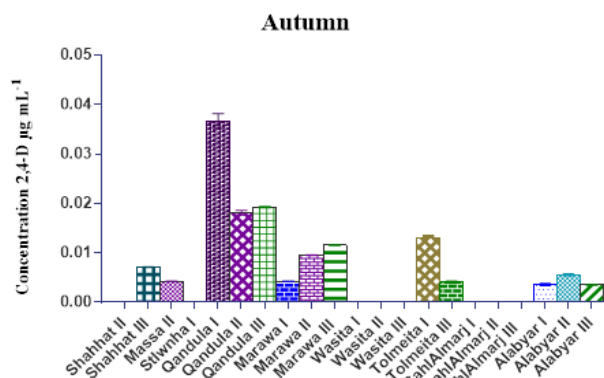


Figure (6). Residues of 2, 4-D pesticides in groundwater from different regions of east Libya during the autumn season of 2016.

Histograms in Figures (7 and 8) show the result of water samples analysis, which were collected from different regions during the winter season 2016. Dicofol, DDD, DDT, DDE, and Heptachlor were not detected according to the device limit.

Results showed that from all surface water, (88.8%) of all samples were contaminated with 2, 4-D residue. Also, (95.23 %) of the groundwater was contaminated during the winter of 2016. However, most of 2, 4-D concentrations in both surface and groundwater sources were under the limit of WHO and LNCSM excluding Wadi Darna[1], Karsa[2], Aldabusia[3], Apouloana, Shahhat I[4], Shahhat II[4], Qandula III[15], Alabyar II[29], and Alabyar III [30] that were higher than MRL's.

In addition, 2,4-D was found in surface water with a concentration ranging from 0.012 $\mu\text{g mL}^{-1}$ (Saylun Massa III)[9] to 0.039 $\mu\text{g mL}^{-1}$ (Wadi Darna) [1] and detected in groundwater with a concentration ranging from 0.018 $\mu\text{g mL}^{-1}$ (Marawa III)[18] to 0.038 $\mu\text{g mL}^{-1}$ (Shahhat II)[4].

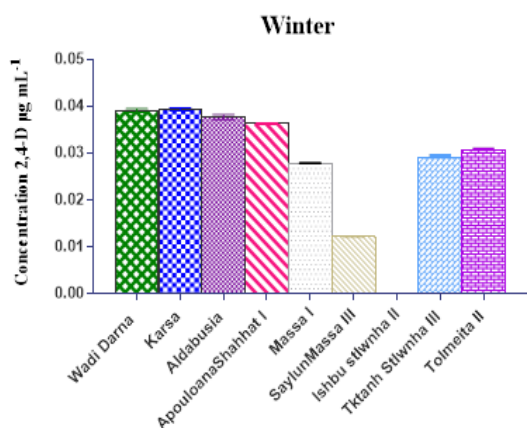


Figure (7). Residues of 2, 4-D pesticides in surface water from different regions of east Libya during the winter season of 2016-2017.

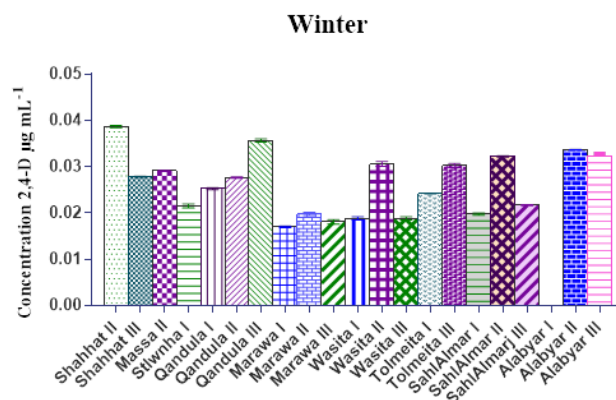


Figure (8). Residues of 2, 4-D pesticides in groundwater from different regions of eastern Libya during the winter season of 2016.

In general, by using an analysis of variance, the 2, 4-D residue in surface water of different regions showed significant differences between Wadi Darna [1] and Karsa[2], Aldabasia[3], Apouloana Shahhat I[4], Saylun, Mass III[9], Tktanh Stlwnha III[12], Tolmeita II[23] in summer, autumn, and winter seasons at ($p < 0.05$). Also, in these seasons, an analysis of variance (ANOVA) for groundwater showed a significant difference between regions at ($p < 0.05$).

In the T-test, a significant difference between surface and groundwater of all analyzed seasons at ($p < 0.05$) was observed in the mean levels of the 2, 4-D residue. Moreover, Analysis of variance (ANOVA) for the concentration of 2, 4-D showed a significant difference in summer, autumn, and winter seasons at ($p < 0.05$).

These results demonstrate that there were certain potential health risks to drinking water in the Wadi Darna [1] region in summer and winter due to the concentration of 2, 4-D residues being higher than MRL's. The high concentration of 2, 4-D in winter from the autumn season in the surface water could be attributed to the agricultural runoff resulting from the extensive agricultural activity around sources of water in

these regions. The surface water was more polluted than groundwater. This was possibly due to the fact that 2, 4-D can reach the surface by the runoff process due to its low soil absorptivity, high potential of leachability, and high-water solubility. The low biodegradability of 2, 4-D in the water also makes 2, 4-D a major pollutant in the surrounding water sources.

From the results of the analysis of variance of all samples collected from all regions within three seasons (summer, autumn, and winter), The maximum contamination has been found to be in summer followed by winter with a minimum in autumn. The probable reason for the higher residues in summer may have been due to using herbicides frequently (Coady et al., 2013).

Finally, the misuse of these pesticides by the individuals concerned and the lack of, or weak, national control plants are behind the presence of these pesticides in water sources and, as a result, the occurrence of such pesticide residues in drinking water represents an environmental and health hazard. A frequent monitoring program is urgently needed in order to assess health risks associated with such contaminants, especially with chronic exposure or a lifelong intake of contaminated drinking water.

CONCLUSION

A quantitative HPLC–UV method has been developed and validated for the analysis of six OCPs using a liquid-liquid extraction (LLE) method. An isocratic HPLC method has been successfully applied to a routine study of the determination of these six OPCs in water or any other sample prior to extraction. A water and acetonitrile mixture (25:75), at a flow rate of 1.0 ml min⁻¹ was found to be an appropriate mobile phase, allowing adequate and rapid separation of six OCPs. This method is simple, accurate, precise, and specific and could separate six OCPs in a mixture solution. Its wide linear dynamic range and low limit of detection

and quantitation make this method applicable for water and formulation analyses of OCPs.

Results obtained in this study from samples collected through selected sites in the eastern region of Libya showed only 2, 4-D residue with a high concentration in summer samples than winter samples followed by the autumn season samples. These differences are probably due to climatic variations and agricultural activity between these areas.

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تطوير وتحقق وتطبيق طريقة على أساس الطور العكسي لجهاز HPLC لغرض التقدير المتزامن لستة أنواع من مبيدات الآفات الكلورية العضوية في عينات المياه السطحية والجوفية المأخوذة من مناطق شمال شرق ليبيا

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المستخلص: تم فحص تلوث المياه بمبيدات الآفات الكلورينية العضوية في مواقع الدراسة في مناطق شمال شرق ليبيا لتقدير الوضع الحالي للتلوث في مصادر المياه السطحية والجوفية. أجريت هذه الدراسة لتحديد تراكيز بعض متبقيات المبيدات الآفات الكلورينية في عينات المياه السطحية والجوفية التي تم جمعها من ثلاثون موقعاً مختلفاً في الجبل الأخضر شمال شرق ليبيا خلال فصول الصيف والخريف والشتاء في عام 2016. تم تطوير طريقة بسيطة وسريعة تعتمد على طريقة استخلاص السائل متبوعة باستخدام جهاز الكروماتوفاي عالي الأداء. مع استخدام العمود ذو الطور العكسي لتحديد ستة مبيدات كلورينية عضوية في عينات المياه في وقت واحد. استخدم في هذه التجربة جهاز مزودة بعمود C_{18} (250 مم × 4 مم؛ 4.6 ميكرومتر حجم الجسيمات) وكاشف الأشعة فوق البنفسجية بطول موجي 238 نانومتر. يتكون الطور المتحرك من 0.1% بروبانول في الماء وأسيونيتريل (75/25، ح / ح). تم التحقق من صحة هذه الطريقة باستخدام محاليل مرجعية لهذه المبيدات في مستويات تراكيز مختلفة، ويظهر منحي خطي جيد في التركيزات بين 5.0 و 25 ميكروغرام لكل مل. حيث تراوحت حدود الكشف وحدود الكمي من 0.71 إلى 2.24 ميكروغرام لكل مل و 2.16 إلى 6.79 ميكروغرام لكل مل، على التوالي. وكذلك تراوح الانحراف المعياري النسبي من 0.026 إلى 0.673%. تبين من النتائج أن متبقيات المبيدات الآفات الكلورينية الوحيدة الموجودة في منطقة الدراسة هي 2,4-D مع اختلاف مستويات متبقياتها خلال الفصول. أظهرت النتائج الإجمالية أن المياه السطحية كانت أكثر تلوثاً بـ 2,4-D عن المياه الجوفية، والتي تم اكتشافها في النطاق الواسع للتركيز من 0.037 إلى 0.385 ميكروغرام لكل مل، 0.003 إلى 0.047 ميكروغرام لكل مل و 0.012 إلى 0.039 ميكروغرام لكل مل خلال الصيف، الخريف والشتاء، على التوالي.

الكلمات المفتاحية: التحقق. الجهاز الكروماتوفاي عالي الأداء. متبقيات. المياه السطحية. المياه الجوفية.