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Effectiveness of QuEChERS Method for the Analysis of Organochlorine Pesticides Residues in Fish

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ABSTRACT

This study examined methods used in the determination of Organochlorine Pesticides (OCPs) in fish. It compared the effectiveness and sensitive of two extraction methods: Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Ultrasound Assisted extraction (UAE) and Accelerated Solvent extraction (ASE) methods in the analyses of 12 OCPs in fish samples. For each method, same sample mass (2 g), solvent volume (10 ml) and sorbent mass (0.1 g PSA and 0.1 C18) were taken. Extracts were analyzed using the gas chromatography electron capture detector (GC-ECD). Both ultrasound-assisted and accelerated solvent extraction methods demonstrated robust validation, with recovery rates ranging from 81% to 99%. Precision was excellent, as evidenced by relative standard deviations (RSDs) consistently below 20%. The detection limits for both methods were comparable, falling between 0.01 and 0.06 µg/ml. Similarly, the limits of quantification (LOQs) ranged from 0.021 to 0.091 µg/ml. The squared correlation coefficient (R^2) ranged from 0.959 to 0. 999, revealing good sensitivity and accuracy. Statistical analysis of real samples determination of OCPs using two tailed T-Test showed that P value is greater than 0.05 (0.084). Therefore, the null hypothesis (Ho) shows there is no significant relationship between Ultrasound Assisted method and Accelerated Solvent Extraction methods. Box plot also shows that Ultrasound Assisted method is more effective and sensitive for small number of samples as compared to Accelerated Solvent Extraction method.

Keywords: QuEChERS, UAE, ASE, GC-ECD, and OCPs

INTRODUCTION

The production of most Organochlorine Pesticides in several countries in the 1970s released considerable levels of these compounds to the environment [1, 2]. Due to their high toxicity, persistence in the environment and lipophilicity, they might accumulate in fat tissues and

biomagnify in trophic levels. These OCPs are very stable in both fresh and salt water, and resistant to photo degradation [3, 4].

Many studies have aimed at detecting these compounds in different environments and matrices. Organisms such as fish are suitable for the monitoring of environmental pollution, since OCPs bioaccumulate easily in their tissues [5, 6]. Different kinds of fish are able to accumulate OCPs in concentrations higher than that of the water due to the diffusion of these compounds through the gills and skin [7]. Monitoring OCPs concentration in fish tissues might be a challenge, mainly due to the low concentration of the target analytes in tissues and high concentrations of interfering substances inherent in the matrix that might affect negatively the analysis results [8].

The selection of a methodology to prepare the sample is highly dependent on the characteristics of the matrix. The main interfering agents in fish tissues are lipids, which can be extracted contiguously to the target analytes [9]. The efficient removal of these compounds from the extract during the sample preparation is crucial to limit the interference and minimize problems of sensitivity and repeatability during the chromatographic analysis [10] thus avoiding the impairment of the detection system, which would make it impossible to obtain reliable results [11]. Several methods have been studied to extract OCPs from fish tissues. These include Soxhlet extraction [12, 13], liquid-liquid extraction (LLE) [14], pressurized liquid extraction (PLE) [15], microwave assisted extraction (MAE) [16], matrix solid phase dispersion (MSPD) [17], accelerated solvent extraction (ASE) [18], ultrasound extraction [19, 20]. Solid phase extraction (SPE) [21, 22], and QuEChERS [23].

The QuEChERS method (Quick, East, Cheap, Effective, Rugged and Safe) was developed by Anastasiades et al. [23]. It became important for the analyses of non-polar chemical residue such as OCPs in fruit and vegetables. Its several advantages include simplicity, low cost and fast application [24]. Recently, the QuEChERS method has been studied with some modifications. Many of these changes originated from the need to obtain better values in the recovery of matrices with high lipid content and with different complexities while avoiding the degradation of analytes [25]. Most of the modifications disregard the amount of sample used in the extraction. It can vary from 5 g [18] to 12.5 g [11].neglecting the need to determine these compounds in samples in which the individual total mass is in some cases below 5 g [26]. Some methods, even employing a small sample mass, require a high consumption of adsorbents and its

application is focused on samples with low fat content, as well as problems with reproducibility [27].

One of the ways of minimizing the use of samples in the OCPs determination in fish tissues is the use of ultrasound-assisted extraction along with the QuEChERS. The ultrasound provides a more efficient contact between the sample and the solvent, due to the increase in pressure, which favours the penetration and transportation, and temperature, which improves solubility and diffusivity, thus favouring the extraction of these compounds. Several extractions can be performed simultaneously and no specialized laboratory equipment is necessary, only the ultrasound bath. Compared to other methods [28], this technique offers a cost-effective and straightforward approach, particularly in samples with high lipid content like fish tissues. On the other hand, accelerated solvent extraction (ASE) offer fast extraction (12-18 min.) with a small amount of solvent (10-40 mL) and a large sample (up to 100 g). In this approach, analytes are efficiently extracted from the sample at high pressure and temperature simultaneously and most of the co extracted compounds are retained in the sorbent. However, ASE equipment is relatively expensive and extraction normally requires a cleanup step. Apart from the method performance, both methods have resulted in new possibilities for sample treatment and such advantages as a reduction of the extraction time, minimal solvent usage and waste, ease of performance without requiring much training or technical skill and a lower usage of lab ware [29]. The ASE method with in-cell cleanup integrates the extraction and cleanup processes in extraction (d-SPE)) provides simultaneous extraction and cleanup [30]. The optimization of these sorbents is also promising regarding modifications in OCPs extraction methods in fish tissues.

Therefore, the aim of this study is to evaluate the efficiency and reliability of the QuEChERS method for extracting and analyzing organochlorine (OCP) residues in fish samples.

MATERIAL AND METHODS

Chemicals and materials

Certified reference chemicals (Individual OCPs: Endrin [END], Dieldrin [DIE] Allethrin, Fenpropathrin, Biefethrin, Lambda-cyhalothrin, Permathrin, Cyfluthrin, Cypermethrin, Fenvalerate and Deltamethrin), used for the identification and quantification were obtained from the National institute of science and technology GmbH (Augsburg, Germany). Reagents used in the study comprised of: acetonitrile (pesticide grade, BDH, England), acetone (pesticide grade,

BDH, England), hexane (analytical grade, BDH, England), ethyl acetate (pesticide grade, BDH, England), toluene (pesticide grade, BDH, England), sodium sulfate (pesticide grade, Aldrich-Chemie, Germany), sodium chloride (pesticide grade, Riedel-de Haen), dipotassium hydrogen phosphate (analytical grade, BDH, England), potassium dihydrogen phosphate (analytical grade, BDH, England), The primary secondary amine (PSA) and octadecyl (C18) were obtained from Varian (Harbor City, USA).

Pesticides stock solutions (1000 µg/mL) of individual OCPs standards were prepared by dissolving 25 mg corrected by purity of the pesticide in 25 mL of ethyl acetate. Pesticide intermediate standard solution (10 µg/mL) was prepared by transferring 250 µL from each pesticide stock solution to a 25 mL volumetric flask and diluting to the mark with ethyl acetate. A mixed standard solution (1.0 µg/mL) containing the twenty-four selected OCPs, indicator was prepared by transferring 1mL of each 10 µg/mL mixed OCPs into a 10 mL volumetric flask and adding ethyl acetate to make up the mark. Several standard solutions from these, with concentrations ranging from 0.005 –1.0 µg/mL, were injected to obtain the linearity of detector response and the detection limits of the chemicals studied.

Sample collection and preparation

Three hundred and sixty samples of Bagrus docmak and Clarias gariepinus (n = 180 each) were randomly collected during the dry and rainy seasons from ten (10) different locations around Gongola River and its dams in Gombe state, Nigeria, as shown on the map. The fish samples were collected in clean plastic containers and stored in an ice chest. The length and weight of the fish were measured using a board and a top-loaded scale (Mettle Toledo). Species were sorted, identified and labeled at the Department of Fisheries, College of Horticulture, Dadinkowa in Gombe, before being transported to the laboratory and kept in a freezer at 20 °C before preparation for analysis. The muscle was removed with a stainless-steel knife, homogenized and stored in clean, sealed glass vials which were kept in the freezer until analysis, The edible part of the samples (50 g) were removed for extraction and purification procedures.

Extraction of pesticides residue by QuEChERS method using ultrasound assisted extraction

The method by Tatiana et al. [23] was adopted and modified. About 2 g of homogenized fish muscle was weighted into 50 ml centrifuge tube and 10 ml mixture of 1:1 v/v of acetone: hexane

added and the mixture was hand shaken for 1 min then, 2 g of Na_2SO_4 was added and hand shaken again for 1 min. Then, 4 g $MgSO_4$ and 1 g of NaCl were added and shaken again for 1 min, and sonicated for 20 min, then centrifuged at 4000 rpm at 4 ^oC for 10 min.

Clean-up of fish extract

About four (4) ml of aliquot of the upper layer was transferred into 15 ml of centrifuge tube containing 0.1 g of PSA, together with 0.1 g C18 and 0.4 g of $MgSO_4$, hand shaken for 1 min and centrifuged at 5000 rpm for 10 min. An aliquot of the supernatant was transferred into auto samples vial and injected into GC- ECD system for analysis.

Extraction of pesticide residue using accelerated solvent extraction.

The method by Haslina et al. [31], was adopted and modified using ASE 350 (Dionex, USA) with 24 ml stainless-steel extraction cell. The cell loading was performed in only one cycle. Two (2) g of homogenized fish muscle was weighted with 2 g of anhydrous sodium sulphate together with earth. The mixture was introduced into extraction cells containing 4 g of $MgSO_4$ and 1 g of NaCl. The extraction was performed using ASE conditions (the extraction temperature, 120 ^0C), extraction pressure, 1500 p.s.I; heating time, 5 min static time; 5 purge time 60 s extraction solvent acetone/hexane (1:1v/v) flush volume 60%).

Clean-up of fish extract

The extract was collected into a vessel. Then, 4 ml of aliquot was taken with 0.1 g of PSA together with 0.1 g of C18 and 0.4 g of $MgSO_4$ was hand shaken and centrifuged at 5000 rpm for 10 minutes, for cleanup and finally, 1 µl transferred into vial for GC-ECD analysis

Determination of OCP in fish extracted using GC-ECD.

The GC-ECD system model, Shimadzu GC-2010 plus withAOC-20 I Auto injector and AOC-20 S Auto sampler, Electron Capture Detector was operated with VF-5-ms (5% phenyl 95% dimethylpolysiloxoane) capillary column that was 30 m long with 0.25 mm internal diameter and 0.25 mm film thickness. The column oven temperature program was 70 ^0C, was held for 2 min and then it was increased to 180 ^0C at 10 min-1 and was held for 1 min and increase from 180 to 300 ^0C at 5 ml min^{-1} and held for 7 min. The detector was at 300 ^0C. Nitrogen was used as a carrier gas with a 1 ml min^{-1} flow. The injection volume was in the split less mode, equipped with automatic sampler and 63Ni-ECD detector.

Method of validation

The method was in-house validated through the following performance criteria: linearity, linear range, sensitivity, limit of detection (LOD), limit of quantitation (LOQ), intra and inter-assay precision and accuracy.

Recovery

The equation below was used to calculate the percentage of the pesticides that were recovered during the analysis. The percentage recovery was calculated as:

$$\% \text{ Recovery} = \frac{amount\ of\ analyte\ recovered}{amount\ of\ analyte\ spilk} \times 100 \quad (1)$$

Figures 1.1 and 1.2 are the phases of the preparation method in Ultrasound assisted extraction

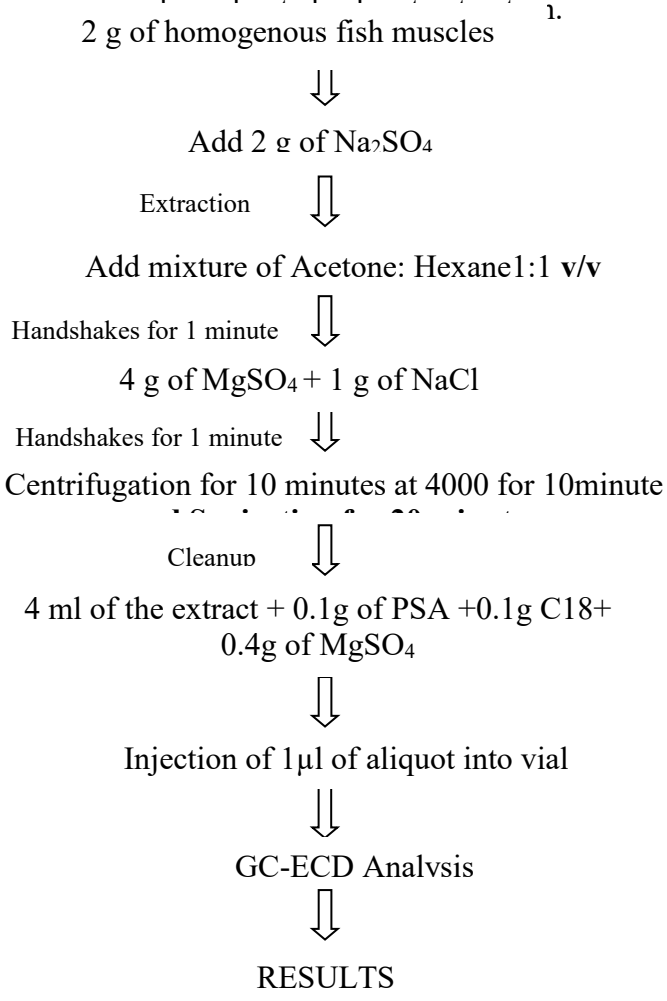

Figure 1.1 Ultrasound Assisted Method

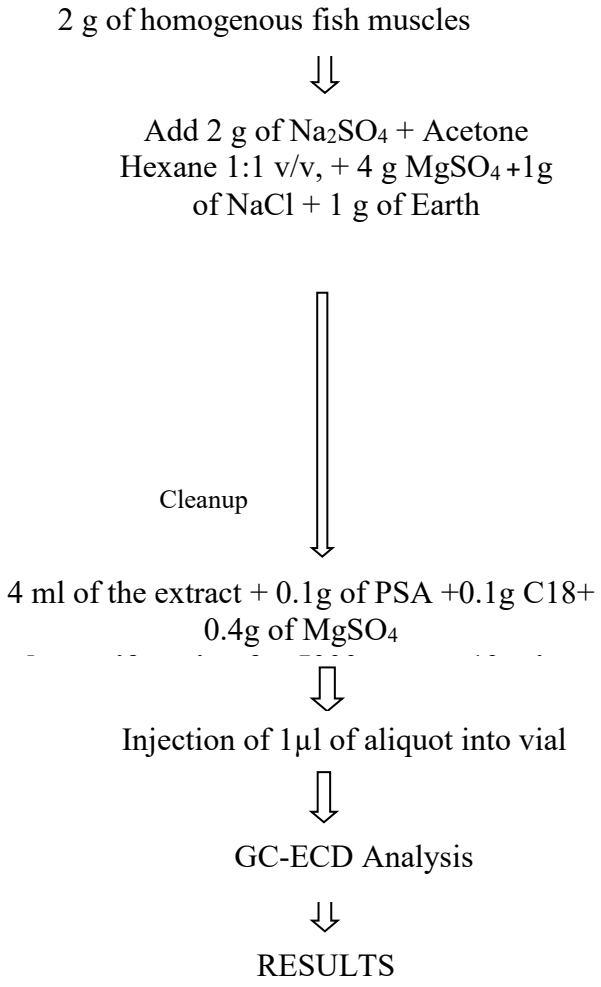

Figure 1.2 Accelerated solvent Method

Table 1: Determination of DRIN via Ultrasound Assisted Extraction Method in ppm

Location	Season	Aldrin	Allethrin	Dieldrin	Endrin	Biefethrin	Fenpropathrin	Permathrin	Lambda	Cyfluthrin	Cypermethrin	Fenvalerate	Deltamethrin
Nafada	Wet	0.25	0.28	0.55	0.06	0.01	0.11	0.26	0.05	0.49	0.18	0.14	0.44
	Dry	0.83	0.22	0.91	0.05	0.01	0.10	0.19	0.08	0.65	0.24	0.35	0.57
Ashaka	Wet	3.05	0.19	0.70	0.11	0.02	0.25	0.18	0.06	0.35	0.08	0.07	0.40
	Dry	0.58	1.26	0.85	0.03	0.02	0.16	0.13	0.07	0.40	0.16	0.18	0.58
Kupto	Wet	2.50	0.39	1.32	0.09	0.09	0.43	0.06	0.51	0.93	0.94	0.31	0.83
	Dry	0.52	0.53	1.40	0.11	0.01	0.24	0.10	0.08	0.75	0.11	0.15	0.35
Zoto	Wet	1.31	0.14	0.76	0.05	0.02	0.11	0.17	0.13	0.20	0.10	0.08	0.19
	Dry	0.26	1.52	0.98	0.20	0.01	0.46	2.60	0.99	4.91	0.80	0.46	0.50
G/west	Wet	0.43	0.26	0.58	0.16	0.00	0.25	1.59	0.23	0.47	0.07	0.09	0.65
	Dry	0.06	0.10	0.99	0.05	0.02	0.06	0.11	0.10	0.59	0.12	0.16	0.23
G/east	Wet	1.56	0.30	0.74	0.09	0.01	0.11	0.16	0.06	13.51	0.03	0.18	0.61
	Dry	0.27	0.14	1.06	0.14	0.01	0.09	0.22	0.05	0.56	0.26	0.35	0.73
Kalgari	Wet	0.05	1.03	1.33	0.17	0.02	0.17	0.48	0.06	0.99	0.04	0.09	0.32
	Dry	0.06	0.24	1.30	0.11	0.01	0.18	0.23	0.33	1.22	0.38	0.21	0.50
Diffa	Wet	1.26	1.15	1.24	0.13	0.04	0.16	0.14	0.05	4.56	0.09	0.21	0.15
	Dry	0.19	0.75	0.79	0.04	0.02	0.09	0.08	0.37	5.12	0.10	0.12	0.43
Dadinkowa	Wet	2.77	0.80	1.82	0.13	0.02	0.09	0.09	0.07	0.47	0.07	0.16	0.46
	Dry	1.35	0.47	0.74	0.10	0.02	0.12	0.28	0.22	0.61	0.57	0.34	0.63
Hinna	Wet	1.28	0.34	0.58	0.10	0.01	0.03	0.17	0.13	6.86	0.05	0.12	0.43
	Dry	0.37	0.09	0.85	0.10	0.02	0.09	0.25	0.05	0.26	0.14	0.14	0.67

Table 2: Determination of DRIN (in ppm) via Accelerated Solvent Extraction Method

Location	Season	Aldrin	Allethrin	Dieldrin	Endrin	Biefethrin	Fenpropathrin	Permathrin	Lambda	Cyfluthrin	Cypermethrin	Fenvalerate	Deltamethrin
Nafada	Wet	2.12	0.12	0.25	0.05	0.01	0.12	0.09	0.01	0.12	0.02	0.01	0.09
	Dry	1.90	0.13	0.37	0.06	0.01	0.15	0.12	0.02	1.08	0.04	0.04	0.13
Ashaka	Wet	0.10	0.15	0.23	0.03	0.01	0.06	0.07	0.03	0.29	0.05	0.05	0.12
	Dry	0.17	0.20	0.07	0.03	0.01	0.06	0.02	0.02	0.30	0.02	0.10	0.07
Kupto	Wet	0.13	0.22	0.61	0.12	0.04	0.44	0.04	0.47	1.13	0.47	0.09	0.12
	Dry	0.16	0.21	0.03	0.02	0.00	0.05	0.02	0.01	0.08	0.01	0.03	0.08
Zoto	Wet	0.17	0.07	0.04	0.03	0.01	0.11	0.04	0.02	0.23	0.05	0.04	0.05
	Dry	0.20	0.11	0.25	0.02	0.01	0.02	0.02	0.01	0.06	0.01	0.02	0.15
G/west	Wet	0.20	0.14	0.06	0.03	0.01	0.01	0.05	0.01	0.06	0.01	0.05	0.06
	Dry	3.56	0.18	0.23	0.03	0.01	0.14	0.08	0.01	0.03	0.01	0.04	0.24
G/east	Wet	0.16	0.05	0.26	0.03	0.01	0.09	0.04	0.02	0.49	0.05	0.05	0.58
	Dry	0.06	1.26	0.35	0.03	0.01	0.07	0.02	0.01	0.68	0.04	0.02	0.20
Kalgari	Wet	3.82	0.28	0.35	0.06	0.01	0.19	0.79	0.03	0.76	0.05	0.04	0.13
	Dry	0.08	0.05	0.28	0.03	0.00	0.05	0.03	0.02	0.10	0.03	0.04	0.08
Diffa	Wet	0.06	0.16	0.31	0.06	0.02	0.04	0.02	0.01	0.03	0.01	0.02	0.07
	Dry	0.08	0.08	0.30	0.04	0.01	0.09	0.04	0.03	0.15	0.03	0.04	0.07
Dadinkowa	Wet	0.13	0.04	0.40	0.02	0.01	0.08	0.05	0.01	0.20	0.04	0.05	0.27
	Dry	0.14	0.05	0.21	0.03	0.01	0.03	0.07	0.02	0.30	0.07	0.04	0.21
Hinna	Wet	0.22	0.06	0.22	0.06	0.01	0.08	0.44	0.07	4.20	0.38	0.02	0.06
	Dry	0.16	0.05	0.25	0.03	0.01	0.05	0.05	0.01	0.18	0.08	0.04	0.06

Table 3: Optimization of the extraction methods

Methods of Extraction	Ultrasound Assisted	Accelerated Solvent
Sample amount (g)	2	2
Type of extraction solvent	Acetone: hexane (1/1 v/v)	Acetone: hexane (1:1v/v)
Sorbent type	PSA: C18	PSA: C18
Extraction steps number	3	1
Sorbent amount (g)	0.1 (PSA): 0.1 (C18)	0.1(PSA):0.1 (C18)
Solvent volume (mL)	10	10
Extraction Time (min)	34 min	14

Development and validation of QuEChERS methods for the analysis OCPs in fish muscles

Aiming at extracting OCPs from fish muscles, a modified QuEChERS method was proposed by employing Ultrasound Assisted Extraction (USAE) and Accelerated Solvent Extraction (ASE) along with GC-ECD using PSA and C18 sorbent for cleaning (figures 1 and 2). Twelve organochlorine pesticides were analyzed in fish samples from the Gongola River and Dadinkowa Dam in Gombe State, Nigeria. Gas chromatography with electron capture detection was employed to quantify pesticide residues. Sample collection and preparation were conducted across various locations and seasons to investigate the potential impact of environmental factors on pesticide contamination

For the OCPs extraction procedures in fish tissue, initially the variables (sample size, type of extractor solvent and sorbent) were evaluated. The objective was to select the most promising solvent and sorbent, as well as to define the sample size necessary for the extraction. The amount of fish tissue used in the OCPs extraction is a conflicting factor in several techniques presented in the literature. Most of them used between 5 [32] and 25 g [33] as last amount for their extraction, such amount is even greater than the total mass certain fish species [26].

Modifications in extraction methods such as the ones obtained in this study might ensure the determination of OCPs in species of small body mass. The mixture acetone: hexane has several suitable characteristics to be used as a solvent in OCPs extraction, mainly because of the Hildebrand/Hansen solubility parameter. This mixture, exhibited Hildebrand solubility parameter

similar to that of the analytes, that is, the analytes were easily solubilized in these solvents that favour their extraction in the matrices under study [34]. This solvent mixture is mainly applied to the extraction of non-polar compounds. It also shows steam pressure and boiling point considered suitable for injection system in gas chromatographers (GC), in addition to being compatible with cleaning phases that use sorbents such as PSA and C18 [35].

Due to these characteristics, the mixture of acetone: hexane 1:1 v/v shows high solubility parameter used in OCPs extraction methods in fish tissue [36]. The PSA and C18 sorbent are the type of sorbent which are also relevant factor in the development of a sample preparation method. The PSA sorbent has an effective chelating effect, bonding with compounds such as free fatty acids and sugars present in the sample, this occurs because the PSA bonds hydrogen with compounds that contain hydroxy or carboxy groups, removing them from the extract. The C18 is a sorbent with structural characteristics that promote its interaction with non-polar compounds in the samples, which are highly used to remove these compounds from samples with fat content. Therefore, the mixture of PSA: C18 has been largely used for the extraction of organic compounds from fish tissues [27, 34, and 36]. Due to the sorbent characteristics mentioned above, the QuEChERS method using ultrasound assisted extraction and accelerated solvent extraction methods with PSA: C18 combined together, provide suitable and excellent sorbents from any interfering agents of different classes present in fish tissues. Therefore, in this first phase of the optimization, the sample size used was 2 g, the mixture of acetone and hexane, and sorbents (PSA: C18) were selected for the study on the optimization of the sample preparation phase. Validation of the method was achieved using blank determination. The percentage recovery ranged between 81% to 95%. Linearity, calibration curve slope and laboratory fortified blank methods were conducted to determine the limits of detection, quantification and %RSD as indicated in Table 1.

The results for USAE and ASE reveal that the limit of quantification (LOQ) varied from 0.021 to 0.05 µg/ml and 0.03 to 0.091 µg/ml, while the limit of detection (LOD) varied from 0.01 to 0.06 µg/ml and 0.01 to 0.04 µg/ml with Relative Standard Deviation (RSD %) below 20% for both techniques used, that is , ranging (3 to 11) and (3 to 9.1) respectively, with the squared correlation coefficient (R^2) ranging from 0.959 to 0.999. Both methods (USAE and ASE) were satisfactorily applied to the analysis of tissue of fish and OCPs residues were detected.

The methods have shown to be effective and sensitive to determine OCPs low concentrations in fish tissues, using small sample mass (2.0 g), and were successfully applied for the analysis of 12 isomers of DRINS class of organochlorine pesticide residues in fish sample analysis.

The results of fish samples analysis of organochlorine pesticides residues obtained from the two different methods, using GC-ECD, with the box plot shown in Figure 2, statistically reveal the distribution of results in the class of OCPs (DRIN,) using the two extraction methods with $\Sigma USAE_{DRIN}$ =127.42 ±26.68, which varied between 2.8 to 17.34, ΣASE_{DRIN} = 43.62±9.22, and ranged between 0.69 to 6.50.

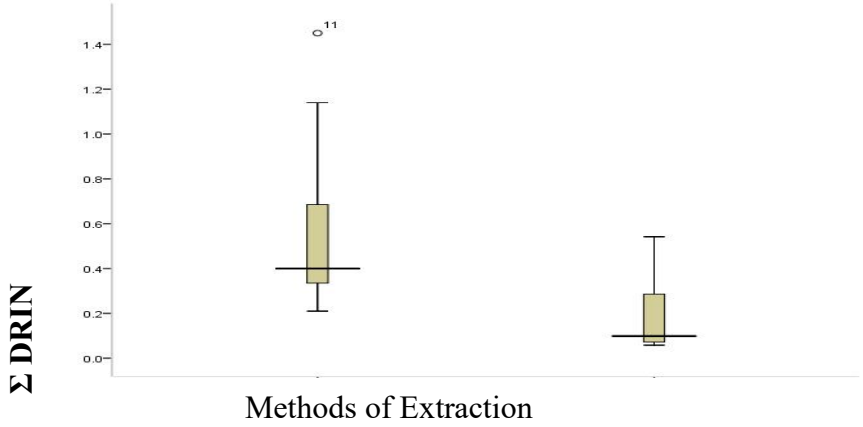

Figure 2: Box plot showing effectiveness of the two methods of extraction

This reveals that the Ultrasound Assisted Extraction method is more effective and sensitive as compared to Accelerated Solvent Extraction method. The results obtained for fish sample analysis by the two different methods were also subjected to statistical analysis using Independent Samples Tests to compare the two sets of data obtained from each method. The results revealed that P value is greater than 0.05 (0.084). Therefore, the null hypothesis (Ho) which states that there is no significant difference between the two sets of data obtained from the two different methods of extraction is accepted, hence there is no significant relationship between the two extraction methods.

The Ultrasound Assisted Extraction method is more effective and sensitive as compared to Accelerated Solvent Extraction method using 2 g mass of the fish muscles based on energy input and matrix interference Therefore Ultrasound Assisted Extraction method is preferred for determination of 12 isomers of Drins class of OCPs in triplicate for the two species of fish in

each of the ten different locations along River Gongola and its dam Dadinkowa Dam and the levels and occurrence of the pesticides residues in fish samples seem to be governed by feeding mode, age and mobility of the biota.

The results of individual isomers of Drins class of OCPs for the two fish species along the ten different locations during wet and dry seasons were summarized in Table 3 using Σ Drins = Aldrin + Endrin + Dieldrin + Allethrin + Fenpropathrin + Biefethrin + lambda-cyhalothrin + Permathrin + Cyfluthrin + Cypermethrin + Fenvalerate + Deltamethrin. The results reveal that Drins isomers in *Claris gariepinus* has Σsum of 117±24.50, with lowest concentration of 2.58 ± 0.71 at Gwani West in the dry season and highest concentration of 13.70 ± 3.72 at Zoto in dry season while *Bagrus docmak* has the Σ sum of 140 ± 29.33, with the lowest concentration of 2.37 ± 0.62 mg/kg in Zoto during wet season and highest concentration of 15.86 ± 4.62 mg/kg at Gwani West during wet season.

This study suggests that *Bagrus docmak* contained higher pesticides residues as compared to *Claris gariepinus* as shown in Figure 3. This contradicts Diego et al [38] and Deribel [39] that reported higher concentration of pesticides residue in *Claris gariepinus* since all the DRINS isomers concentrations exceeded 0.1 mg/kg which is the WHO/FAO limit except few isomers in some locations.

Figures 4 and 5 show percentage distribution of DRINS isomers in *Claris gariepinus* and *Bagrus docmak* during wet and dry seasons along the ten different locations in River Gongola and its dam, Dadinkowa dam.Tthe results obtained demonstrated that the method is suitable to determine OCPs in fish tissues, enabling a thorough evaluation of the environmental contamination on an individual basis, which is a promising characteristic mainly in the toxicological area.

Table 3: Σ DRINS concentrations (mg/kg) in Clarias gariepinus and Bagrus docmak

Location	**Season**	**Σ *Drins* $_{Clarias\ gariepinus}$ ± SD**	**Σ *Drins* $_{Bagrus\ docmak}$ ± SD**
Nafada	Wet	2.82±0.74	3.65±0.97
	Dry	4.17±1.10	3.88±1.01
Ashaka	Wet	5.46±1.60	6.62±1.76

	Dry	4.40±1.18	4.91±1.30
Kupto	Wet	8.40±2.24	11.43±3.14
	Dry	4.34±1.17	5.52±1.55
Zoto	Wet	3.26±0.90	2.37±0.62
	Dry	13.70±3.72	3.19±0.87
G/west	Wet	4.78±1.28	15.86±4.62
	Dry	2.58±0.71	12.85±3.64
G/east	Wet	7.34±2.10	14.08±4.25
	Dry	3.88±1.03	9.74±2.63
Kalgari	Wet	4.74±1.28	3.82±1.02
	Dry	4.76±1.28	6.93±1.87
Difa	Wet	9.17±2.64	3.86±1.01
	Dry	8.10±2.47	9.46±2.95
Dadinkowa	Wet	6.94±1.95	5.50±1.51
	Dry	5.44±1.43	5.31±1.45
Hinna	Wet	10.11±3.16	5.38±1.50
	Dry	3.04±0.81	5.94±1.58

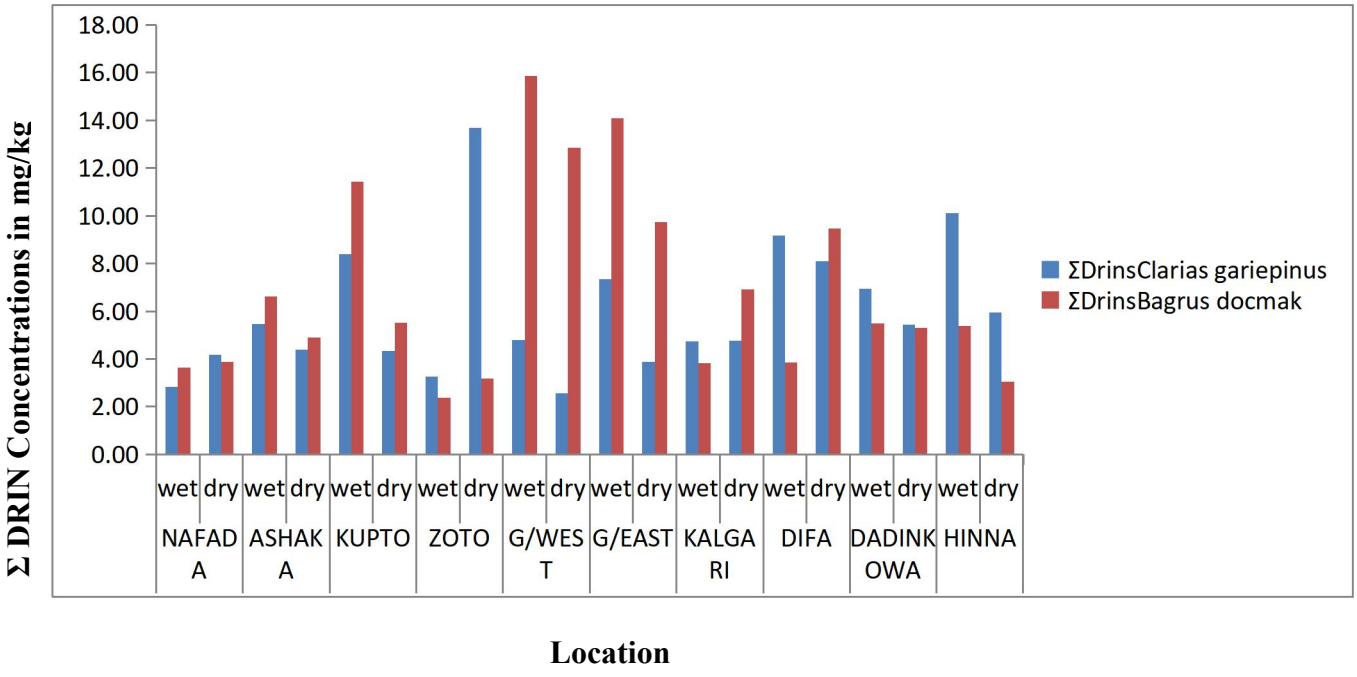

Figure 3: Variation in Concentration of DRINs

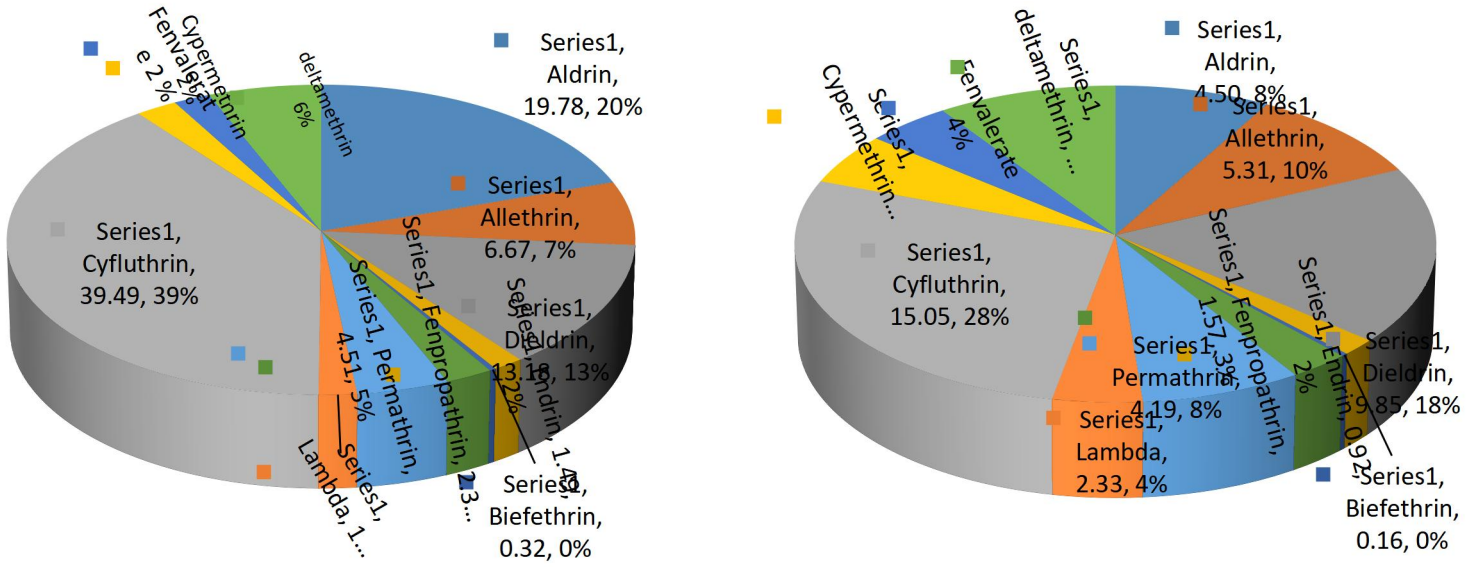

Figure 4: Percentage distribution of Drins in *Clarias gariepinus* wet and dry season

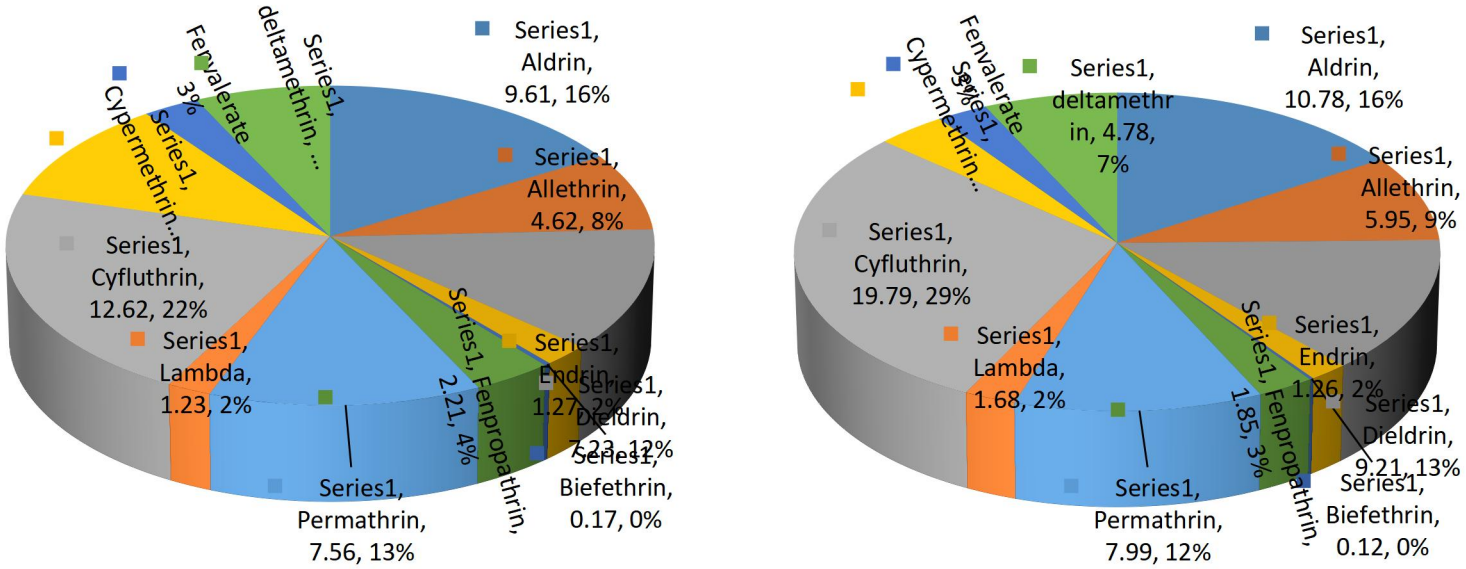

Figure 5: Percentage distribution of Drins in *Bagrus docmak* during wet and dry season

CONCLUSION

The QuEChERS method using both Ultrasound Assisted Extraction and Accelerated Solvent Extraction techniques are both, simple, fast to develop and enabled the extraction of several samples simultaneously in real samples analysis, demonstrated good recovery. Comparing the two methods using Independent Samples Test and Box plot, the methods showed satisfactory results in the OCPs extraction through the recovery and accuracy tests, reaching suitable LOQs,

LODs, RSD and R^2 values. The results revealed that, ultrasound assisted extraction method is more effective and sensitive for small number of samples as compared to Accelerated solvent extraction method. Its main advantage is in the use of small sample size (2 g), which allows the use of tissues of fish of small body mass. Also, it requires the use of small amounts of solvent (10 mL) and sorbents (0.1 g of PSA, 0.1 g of C18, 0.4 g of $MgSO_4$). The results proved that, due to the fast extraction and low material consumption, the methods can be used in routine analyses to determine OCPs residues in fish muscles

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