

APPLICATION OF MODIFIED-QUECHERS METHOD TO FISH TISSUES FOR THE DETERMINATION OF ORGANOCHLORINE PESTICIDES BY GAS CHROMATOGRAPHY, WITH OPTIMISATION USING ¹⁴C-LINDANE AND ¹⁴C-DDT

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ABSTRACT: *In this study, Ethyl acetate modified-QuEChERS method has been applied to fish matrix for the determination of recommended target organochlorine pesticides by keeping in view the importance of fish as food and an important indicator of sediment contamination and water quality problems. Fish and shell-fish monitoring facilitate effective risk management practices for responsible agencies and competent authorities by determining levels of contaminants that may be harmful to human consumers. A simple, rapid and inexpensive method has been proposed for the analysis of organochlorine pesticides in fish tissues. The method has been adapted from a previously validated method in IAEA laboratories, for pesticide residues in fruits and vegetables using ethyl acetate extraction, dispersive solid phase clean-up and gas chromatographic analysis with ECD and NPD detection. The method has been validated on fish fillets at fortification levels 10, 100 and 1000 µg/kg levels, far below the Codex permissible limits in fish tissue. Average recovery obtained for all 12-pesticides at three fortification levels is 90% with relative standard deviation of 8% (n=479). Two radiolabelled compounds, ¹⁴C-lindane and ¹⁴C-DDT, were used in the initial stages of method optimization and characterization. Limits of detection (LOD) were less than 3 µg/kg for all analytes except dieldrin, which had a LOD of about 5 µg/kg. The method offered is proven to provide efficient recoveries and most sensitive detection limits.*

KEYWORDS: Modified QuEChERS Method, Organochlorine Pesticides, Fish, GC-ECD, Radiotracer Technique, ¹⁴C-lindane and ¹⁴C-DDT.

INTRODUCTION

The aquatic environment is subject to an ever increasing range of man-made (anthropogenic or xenobiotic) pollutants, reflecting the evermore rapid innovations of our technology to manufacture goods to satisfy a perceived increase in consumer demand on which our economy is based. These artificial organic compounds introduced a revolution in the industrial and agricultural sector from the second half of the past century. But these bioaccumulative pollutants after production or after use in their respective fields, whether released into atmosphere, onto land or into the rivers eventually come to rest in the aquatic ecosystem. Since fish as inhabitant of the rivers, lakes and oceans are, perhaps, the class of vertebrate most at risk of exposure to these pollutants. (Michael et al. 1999)

Most of these organic pollutants i.e. organochlorine pesticides, chlorinated biphenyls, PAHs, phenols and phthalates are evident to have potential endocrine disrupting effects. These

chemicals entering to the estuarine, river or sea are mainly targeted to and bioaccumulate in fish and marine mammals to relatively high concentrations more than 10, 00,000 times the concentration detected in the water column. (US EPA 2000; Krahn et al. 2005). Fish act as nonpolar media that can adsorb hydrophobic organic chemicals within the water column; this makes fish good biomonitors for xenobiotic pollutants. Since birds and humans consume fish, ingestion of foods contaminated with persistent lipophilic pesticides and PCBs can result in the accumulation of these pesticides in humans may leading to reproductive failures, birth defects, immune system dysfunction, endocrine disruptions, and cancers. (Kasozi et al. 2003).

For the importance of issue there is always a need for affordable analytical methodologies for monitoring of these persistent organic pollutants in marine fishes for responsible and possible health risk management practices. Analytical methods for the analysis of these contaminants are widely available and are a result of vast amount of environmental analytical method development research on POPs over the past 30-40 years (Muir et al. 2006). However, these employ complex, time consuming and expensive extraction, clean-up and analytical procedures and are difficult or impossible to apply in many developing countries.

IAEA modified QuEChERS-ethyl acetate method after its successful application to agricultural products has been now applied and validated for fish matrix for the determination of organochlorine pesticides. Although a number of these compounds are banned in most parts of the world since almost four decades but because of their high persistence, residues of these compounds are still detectable in fish tissues in different regions of the world (Lisa Hoferkamp et al. 2010), also because of their high efficacy and lower cost these compounds are still in use in several developing countries and these compounds have ability to undergo long distance atmospheric transport (Kasozi et al. 2003).

The IAEA-ethyl acetate multi residue method for the determination of pesticide residues in fruits and vegetables is an adaptation of the QuEChERS method (Anastassiades et al. 2003). Ethyl acetate is used for the extraction to reduce costs and permit analysis by gas chromatographic (GC) techniques using conventional electron capture and nitrogen-phosphorous detectors (ECD, NPD) as well as mass spectrometric detection, in order to increase the applicability of the method to laboratories where mass spectrometry is not available (Aysal et al. 2007). IAEA-Ethyl acetate QuEChERS method has been adopted for determination of EPA recommended target organochlorine pesticides for fish (Table 1) (USEPA. 2000), with slight modification in extraction procedure and followed by dispersive solid phase extraction cleanup with primary-secondary amine sorbent and anhydrous magnesium sulphate to remove many polar matrix components common in food matrices, such as organic acids and certain polar pigments. During development/adaptation, the individual steps of the method were optimized using radiolabelled versions of a relatively non-polar and a polar organochlorine pesticide, ^{14}C -lindane and ^{14}C -DDT, respectively, to guarantee the method effectiveness. The method was validated using spiked samples of Nile perch, which is one of the recommended predator target species for inland fresh water and great lake waters.

METHODOLOGY

Chemicals and Reagents

The 12 pesticide reference standards consisted of hexachlorobenzene, lindane, heptachlor, aldrin, dieldrin, endrin, dicofol, alfa-endosulfan, beta-endosulfan, *p, p*-DDT, *p,p*-DDD and *p,p*-DDE were all near-100% purity, obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). IUPAC names for these pesticides are given in Table 1. Stock solutions of 1 mg/mL and working solutions containing the 12 pesticides at 750, 7500 and 75000 pg/ μ l for the three fortification levels were prepared in 85:15 (v:v) acetone:isooctane. Mixtures of “cold lindane (Dr. Ehrenstorfer) and ^{14}C -lindane” and similarly “cold DDT and ^{14}C -DDT” to make a total cold pesticide concentration of 3000 pg/ μ l were separately prepared in acetone. Each spike contained approximately 60,000 dpm activity of ^{14}C -lindane or ^{14}C - DDT in 500 μ l to fortify 15 g sample yielding approximately 2,000 dpm/mL activity in the 30 mL EtOAc extract. ^{14}C -pesticide recoveries were measured by LSC in different sequences (runs).

All organic solvents used in the study were pesticide or HPLC grade. High purity anhydrous Na_2SO_4 was obtained from Merck, and anhydrous MgSO_4 ($\geq 98\%$ purity) was from Fluka. The MgSO_4 was baked at 500°C for 5 h in a furnace to remove phthalates. Primary secondary amine sorbent of 40 μm particle size was obtained from Varian (Harbor City, CA; USA). Perkin Elmer Ultima gold liquid scintillator solution was used for the radioassay in LSC.

Frozen Nile perch fillets were purchased from a local store, and analysed to ensure that they were negative for target analytes. These were used for fortification experiments and to prepare matrix blanks for matrix-matched calibration standards.

Apparatus

Equipments / apparatus used in the study included Agilent 6890 Gas Chromatograph, a Stephan UM 5 universal chopper to comminute fish samples; an UltraTurrax with T25 head to homogenize samples during extraction; a Beckman LS 6000 TA liquid scintillation counter (LSC) to measure radioactivity in the samples fortified with radiolabeled lindane and DDT; a Sigma 4 K15 centrifuge to centrifuge the 50 mL extraction bottles and 20 mL conical glass tubes; a Labinco L46 vortexer to shake the dispersive-SPE tubes; Sartorius CP 225D analytical and top loading balances to weigh standards, salts and samples.

For extraction, 100 mL round bottom pyrex centrifuge bottles (Cole–Parmer A-34533-01) were employed, and 20 mL glass conical Zymark tubes were used for dispersive-SPE cleanup. For LSC, 20 mL polyethylene vials were utilized.

Preliminary steps

Partially frozen Nile perch fillets of 1 kg were comminuted using the Stephan chopper. Homogenous samples were divided into 100 g portions and stored in a deep-freezer at -20°C till the analysis. Composite samples were rehomogenized with a hand blender prior to weighing out analytical portions. A number of 40 mL vials containing 15 ± 0.1 g anhydrous Na_2SO_4 were prepared separately in advance and were stored capped at room temperature until needed in experiments. For dispersive-SPE, 0.25 ± 0.01 g PSA sorbent and 1.5 ± 0.1 g anhydrous MgSO_4 were weighed and mixed into 20 mL conical glass centrifuge tubes and stored capped at room temperature until needed.

Total oil content and pH value of the fish samples were determined as $0.28\% \pm 0.05$ and 6.99, respectively. Since original sample pH is neutral, the sodium bicarbonate (NaHCO_3) which was used as neutralizing agent in original method for fruits and vegetables and cereals, was omitted for the analysis of fish samples.

Extraction and Cleanup

Fish analytical portion (15 ± 0.1 g) of previously comminuted sample of Nile perch were weighed into centrifuge bottle. The samples were fortified with the appropriate 12-pesticides solution (200 μL) to yield 10, 100 and 1000 $\mu\text{g}/\text{kg}$ concentrations. 7 replicates at low and mid levels and 6 replicates at high level (*i.e.* total 20 spiked samples in one occasion) were prepared in two different occasions. The fortified samples were allowed to stand for 30 min for the pesticides to interact with the matrix and some of the solvent to evaporate. Anhydrous Na_2SO_4 (15 g) were mixed with the samples. EtOAc (30 mL) at 30°C was added, and immediately, each sample was extracted with the probe blender for 2 min. The tubes were centrifuged for 3 minutes at 2,500 rpm. Further aliquots (10 mL) of the EtOAc extract were transferred into the dispersive-SPE cleanup tubes (PSA+ MgSO_4) and vortexed (45 s). The tubes were centrifuged for 2 minutes at 1,900 rpm. Finally, the extracts from dispersive SPE tubes were treated further as, the low-spike (10 $\mu\text{g}/\text{kg}$) extracts were concentrated 2-fold using Turbovap, while mid-spike (100 $\mu\text{g}/\text{kg}$) extracts were injected directly, and high-spike (1000 $\mu\text{g}/\text{kg}$) extracts were diluted 5-times with ethyl acetate, transferred to the vials and injected to GC.

Same experiment was performed with ^{14}C -Lindane and ^{14}C -DDT for radiotracer technique. The samples were fortified with 500 μL ^{14}C -lindane solution and ^{14}C -DDT in different occasion to yield 100 $\mu\text{g}/\text{kg}$ concentration and $\approx 2,000$ dpm/mL activity in the 30 mL EtOAc extract. After each step of procedure, like extraction and clean-up, replicate portions of 1 mL were transferred from each extract supernatant to scintillation vials for radio-assay. Scintillation cocktail (12 mL) was added and the activity measured on a LSC to determine the efficiency and repeatability of the extraction and clean-up steps separately. For each batch of samples, a matrix blank and reagent blank (15 mL of deionized water) were also analyzed without any fortification.

GC- analysis

The samples were analysed with Agilent 6890 GC (Little Falls, DE; USA) using HP-5 capillary column of 30 m, 0.25 mm i.d., and 0.25 μm film thickness. The GC-ECD was equipped with split/splitless injector, which was used in the splitless mode. Injector temperature was 250°C and pressure 21.65 psi with a purge flow of 15 ml/min and 0.75 min purge time. The ECD temperature was 300°C with 6 mL/min anode purge flow and 60ml/min combined flow. Helium was used as carrier gas with constant flow at a flow rate of 2 mL/min. Injection volume was 1 μL and the oven temperature program was set at 70°C for 1 min, ramped to 160°C at $20^\circ\text{C}/\text{min}$, a $4^\circ\text{C}/\text{min}$ ramp to 230°C and followed by a $25^\circ\text{C}/\text{min}$ ramp to 280°C held for 7 min.

Quantification was performed using external calibration with matrix-matched standards, which entailed preparing blank extracts for use as the solvent in calibration solutions. The standard solutions were prepared at 3, 7.5, 15, 30, 60, 90, 180, 360 and 540 $\text{pg}/\mu\text{L}$ concentrations for ECD. The matrix equivalent concentration in the calibration standards was 0.5 $\text{mg}/\mu\text{L}$ as in fortified sample extracts injected to GC-ECD.

RESULTS AND DISCUSSION

Homogenization of the sample and extraction:

The first step in the laboratory when conducting an analysis is to comminute and homogenize the sample collected in the field so that a reasonable and representative sub sample can be extracted. To minimize wasted time, effort, cost and reagents in the analytical method, the smallest possible sub-sample should be taken that achieves accurate results for the original sample (Maestroni et al. 2000). Also, efficiency of extraction by shaking requires that the sample be finely chopped to increase accessibility of the solvent to the pesticides within the sample. To remove these issues as a factor, sample has been homogenized / mixed several times, startlingly whole fillets of fish have been comminuted using Stephen chopper, then these composite samples were re-homogenized with a hand blender prior to weighing analytical portions, in last during extraction weighed analytical portion was homogenized with ultra turrax probe blender, to guarantee that the solvent would reach any pesticide encased in the sample.

In original method NaHCO_3 has been used to give consistent pH to the sample during the extraction, because different types of samples have different pH which can affect the recoveries of pH-susceptible pesticides and their stability in the extracts (Aysal et al. 2007). Since fish matrix is found neutral, as its pH value is determined as 6.99, no NaHCO_3 was used as neutralizing agent.

Sodium sulphate (Na_2SO_4) has been used as in the original method for the same purpose of drying agent to increase recoveries of polar compounds in multiresidue method procedures. The drying agent also serves as a dispersant to increase surface area for sample exposure to the solvent and to minimize the amount of free water to interact with the solvent. Hence, same ratios have been used; 1:1 (w:w) sodium sulphate to sample and 2:1 (v:w) EtOAc to the sample.

^{14}C -lindane and ^{14}C -DDT recoveries

When possible, the use of isotopic tracers is an exceptional approach to follow the pathway of a substance through a chemical, physical, or biological system. The unique advantage of radioisotopes is that their behaviour in a system is usually identical with that of their stable counterpart, and they can be identified easily with very high sensitivity by their characteristic radiation, even in unclean extracts (FAO/IAEA 1991). Taken advantage of IAEA laboratories, radiotracers i.e. ^{14}C -lindane and ^{14}C -DDT were used to measure the recovery of individual extraction and cleanup step of the method.

As described in Materials and Methods, ^{14}C -lindane and ^{14}C -DDT were applied at a consistent concentration to all samples. Table 2 shows the recoveries of ^{14}C -lindane and ^{14}C -DDT during the extraction and dispersive-SPE clean-up steps, which gave overall recoveries of 82 and 79% for ^{14}C -lindane and ^{14}C -DDT, respectively, with good precision between 2 and 9 % relative standard deviation (RSD). The dispersive-SPE step did not cause any significant loss of these two analytes in fish.

Fortified Pesticides Recoveries

As described in Materials and Methods, pesticide mixture solution (containing 12-pesticides) was applied in fish at three fortification levels; with 200 μl of 750, 7500 and 75000 pg/ μl to

yield 10, 100 and 1000 $\mu\text{g}/\text{kg}$ concentrations respectively, in appropriate analytical portion of fish which produces respectively 5, 50 and 500 $\text{pg}/\mu\text{l}$ of each analyte in final 1ml extract. Appropriate matrix matched calibrators were prepared accordingly around this final extract's spiked concentration between 50 and 120% expected recoveries.

Figure 1 presents the GC-ECD chromatograms of the analyses for calibration standards of the stated concentrations and fortified extracts at three different levels in matrix and matrix blanks for Nile perch. In chromatograms individual peaks are identified with their retention times. Peak shapes and resolution for all analytes were acceptable and the matrix extract exhibited no interfering peaks. All analytes could be quantified at concentrations $\leq 10 \mu\text{g}/\text{kg}$.

Individual pesticide recoveries of the replicates for different levels in fish were calculated on wet weight basis using weighted linear regression curve. To check for suspected outliers, the Dixon Test was performed (Miller and Ambrus. 2000) and in all, only 1 outlier was removed from the data set of 480 results.

Figure 2 shows the recoveries for all spiked pesticides at each level in fish. The values depicted are averages of results from two different occasions. The accuracy (recovery) and precision (intra-laboratory repeatability, RSD) of the method, averaged for all twelve analytes, are summarized in Table 3. For all 12 pesticides in fish at 3 levels, the overall recovery of the method was 90% with a RSD of 8% ($n = 479$). All pesticide recoveries fell within the acceptable limits recommended by Codex Alimentarius (FAO/WHO, 2003). Recovery for individual analyte at each fortification level is within the 72-100 % range, presented in Table 4.

Limit of Detection (LOD)

In method validation experiments, LOD is another important parameter to be determined for all target analytes. The LOD of the method for each analyte was estimated using matrix-matched weighted-regression calibration curves. Limits of detection for the analytes for GC-ECD analysis were within the ranges of LODs of contemporary published methods for organochlorines in fish, including those using advanced analytical techniques such as mass spectrometry and tandem mass spectrometry (Bienvenida Gilbert-López et al. 2010; Concha-Grana et al. 2010; Schenck Frank et al. 2009; Shubing Chen et al. 2008, US-EPA) . The standard deviations of relative y (response) residuals (S_{rr}) of the weighted regression calibration were ≤ 0.1 for all analytes in the study, thus meeting accepted quality control criteria. LOD values of analytes at different fortification levels are summarized in Table 4. Typical LOD range was 0.4 – 4.5 $\mu\text{g}/\text{kg}$ using GC-ECD.

Uncertainty

As a part of method validation and also requirement of ISO-17025, uncertainty was also estimated. Analysis uncertainty and lab uncertainty have been measured as overall bias (recovery) of the method and overall relative standard deviation of the method respectively according to the recommendations from Eurachem/CITEC Guide (Eurachem Quantifying uncertainty, 2000). The method was rugged with $<10\%$ measurement uncertainties.

Associated with measurement uncertainty (precision), the Horwitz ratio (HorRat), a normalized performance parameter for the acceptability of methods of analysis with respect to intra laboratory precision was also calculated for all pesticides at three fortification levels. Horwitz ratio (HorRat) is the ratio of the observed relative standard deviation calculated from

the actual performance data, RSD_R (%), to the corresponding predicted relative standard deviation calculated from the Horwitz equation $PRSD_R$ (%) = $2C^{-0.15}$. As shown in Table 5, for QuEChERS method for fish HorRat values are within the acceptable range of 0.3-1.3. (Banerjee et al. 2008; Horwitz et al. 2006; HorRat for SLV. 2004)

CONCLUSIONS

The IAEA-modified QuEChERS method using EtOAc at 30°C as extractant and GC-ECD for analysis with slight modification was successfully validated for 12 recommended target organochlorine pesticides in fish tissues at three fortification levels.

This method is a very useful alternative to other published methods for the analysis of organochlorine pesticide residues in fish because of its simplicity and cost-efficiency. It can be applied using laboratory apparatus and gas chromatographic instrumentation available in most pesticide laboratories, including those in developing countries. Ethyl acetate is a less expensive solvent than acetonitrile, which is used in the original QuEChERS method, and this cost advantage will become even more marked because of the predicted world shortage of acetonitrile in future and the consequent increase in the price and difficulty in sourcing this solvent. The analyte list could also be extended to other compounds like organophosphorus pesticides, PCBs and PAHs in different matrices to make the method a useful tool for fish and shellfish contaminant monitoring programs.

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APPENDIX

Table 1. EPA Recommended target analytes (organochlorines) for fish and shell fish contaminant studies

Organochlorine pesticides	This study	IUPAC Name
Chlordane, total (cis- and trans-chlordane, cis- and trans-nonachlor, oxychlordane)		-----
DDT, total (<i>o,p'</i> -DDD, <i>p,p'</i> -DDD,	<i>p,p'</i> -DDD	1,1,1-trichloro-di-(4-chlorophenyl)ethane
<i>o,p'</i> -DDE,	<i>p,p'</i> -DDE	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
<i>p,p'</i> -DDT)	<i>p,p'</i> -DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
Dicofol	Dicofol	2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol
Dieldrin	Dieldrin	(1R,4S,4aS,5R,6R,7S,8S,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octa=hydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
alpha-Endosulfan	alpha-Endosulfan	(1,4,5,6,7,7-hexachloro-8,9,10-trinitroborn-5-en-2,3-ylene=bismethylene) sulfite
beta-Endosulfan	beta-Endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro=6,9-methano-2,4,3-benzodioxathiepine 3-oxide
Endrin	Endrin	(1R,4S,4aS,5S,6S,7R,8R,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
Heptachlor epoxide	Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Hexachlorobenzene	Hexachlorobenzene	Hexachlorobenzene
Lindane	Lindane	1,2,3,4,5,6-hexachlorocyclohexane
Mirex		-----
Toxaphene		-----
	Aldrin	1R,4S,14aS,5S,8R,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro=1,4:5,8-dimethanonaphthalene

* List contains more other contaminants, here only study relevant are presented

Table 2. ^{14}C -lindane and ^{14}C -DDT recoveries and precision (RSD) for extraction and clean-up steps.

^{14}C labelled pesticide	Extraction ^a		Cleanup ^b		Overall ^c	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
	(%)	(%)	(%)	(%)	(%)	(%)
^{14}C -Lindane	90	2	91	2	82	2
^{14}C -DDT	86	9	92	4	79	9

^a triplicate aliquots and measurements of 8 samples in two different days (5+3) for lindane and 7 samples for DDT; recovery for extraction step only

^b duplicate aliquots and measurements of samples; recovery for cleanup step only

^c combination of extraction and cleanup measurements

Table 3. Overview of performance characteristics (accuracy and precision) of the method at different levels (n = 84 for each level).

	Fortification level ($\mu\text{g}/\text{kg}$)	Accuracy	Codex acceptable range	Precision	Codex acceptable range
		Average recovery (%)		Average Repeatability RSD (%)	
Day 1	10	84	70-120	12	20
	100	89	70-120	6	20
	1000	95	70-110	9	15
Day 2	10	83	70-120	10	20
	100	96	70-120	6	20
	1000	91	70-110	6	15
Overall		90		8	

Table 4. Mean recoveries and detection limits of current analytical methods and QuEChERS-EtOAc method for recommended target analytes

Compound	10µg/kg		100µg/kg		1000µg/kg		Limits of QuEChERS EtOAc Method for Fish (µg/kg)	Range of detection Limits of Current Analytical Methods for fish [EPA-2000] (µg/kg)
	Reco	RSD	Reco	RS	Reco	RSD		
	very (%)	(%)	very (%)	D (%)	very (%)	(%)		
Hexachlorobenzene	82	5	95	5	88	4	0.9	0.1-2.0
Lindane	83	6	90	5	89	6	0.4	0.1-5.0
Heptachlor	83	9	93	3	90	5	0.4	0.1-5.0 (heptachlor epoxide)
Aldrin	95	14	86	3	97	5	0.9	-
Dicofol	82	10	94	18	84	11	2.1	1.0-5.0
alpha-Endosulfan	87	9	93	3	90	5	1.3	5.0-10
Dieldrine	78	5	89	5	97	5	4.5	0.1-5.0
pp-DDE	80	5	94	3	100	5	0.5	0.1-38
Endrin	87	11	94	4	94	6	0.5	0.1-15
Beta-Endosulfan	84	5	94	3	92	8	0.5	5.0-70
pp-DDD	82	7	93	3	97	6	0.6	0.1-10
pp-DDT	82	11	93	5	96	6	0.5	0.1-13

Table 5. Horwitz ratio (HorRat) for IAEA-EtOAC QuEChERS method for fish

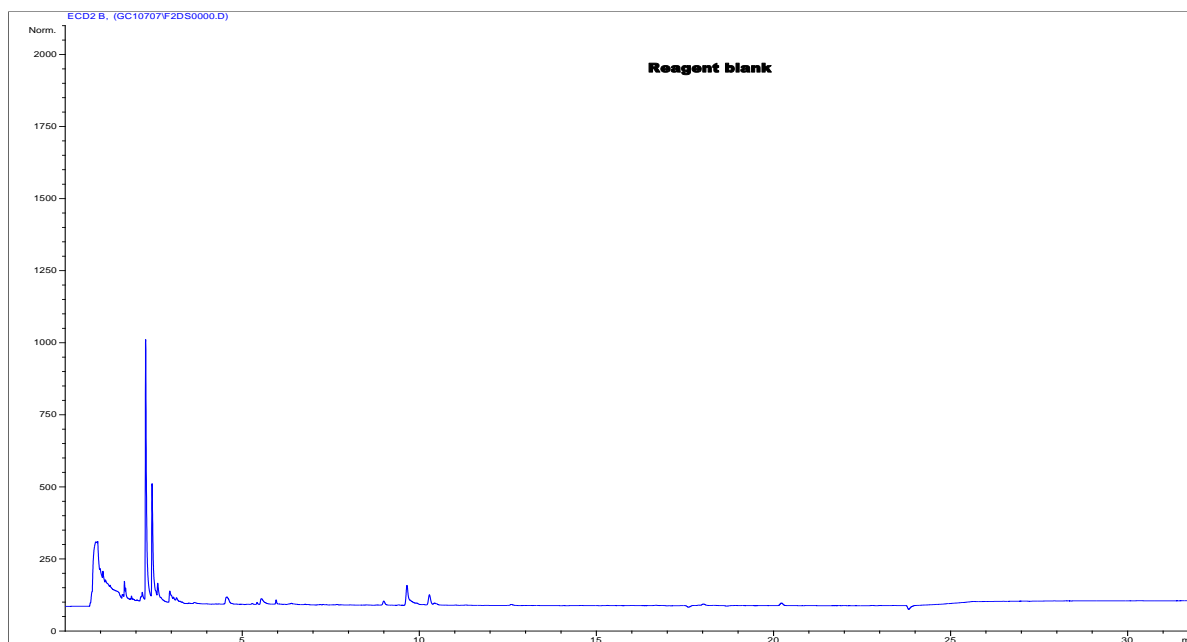
Fortification Level	RSDr	Mass fraction ^a "C"	PRSD ^b	PRSDr ^c	Horwitz Ratio (HorRat)	Acceptable HorRat values for intralaboratory studies
10µg/kg	10.41	0.00000001	32	16	0.66	} 0.3 - 1.3
100µg/kg	6.86	0.0000001	22	11	0.61	
1000µg/kg	5.80	0.000001	16	8	0.73	

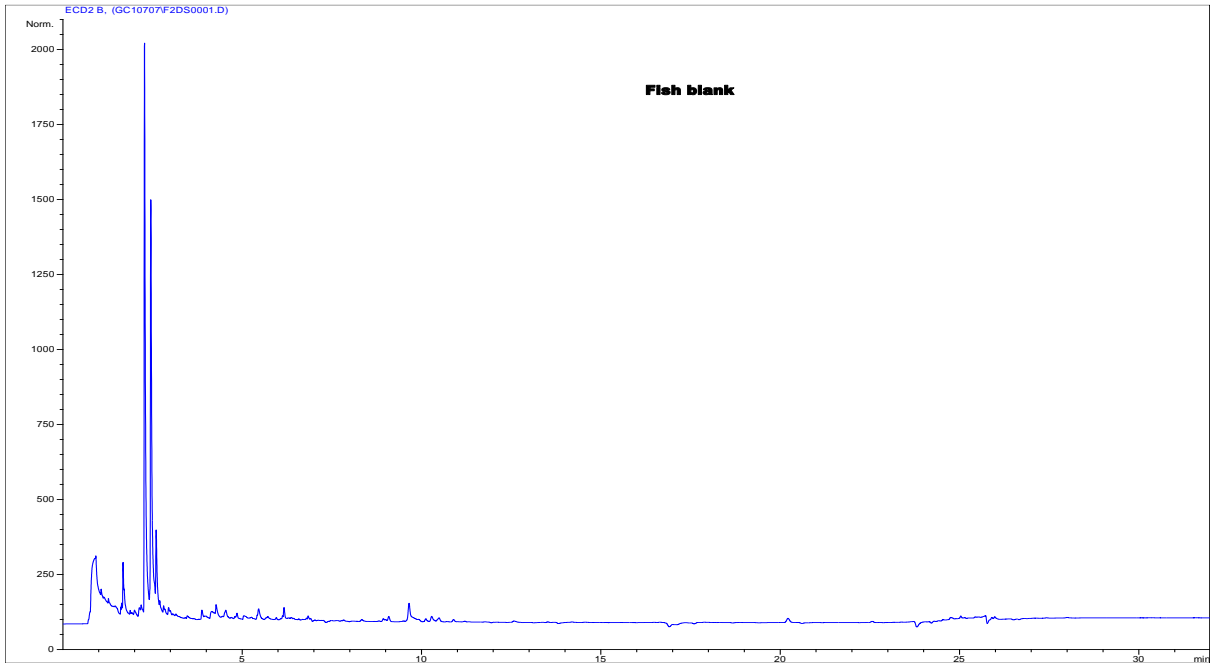
^a Added concentration expressed as a decimal fraction in which units of numerator and denominator are the same.

^b For among-laboratory precision (reproducibility)

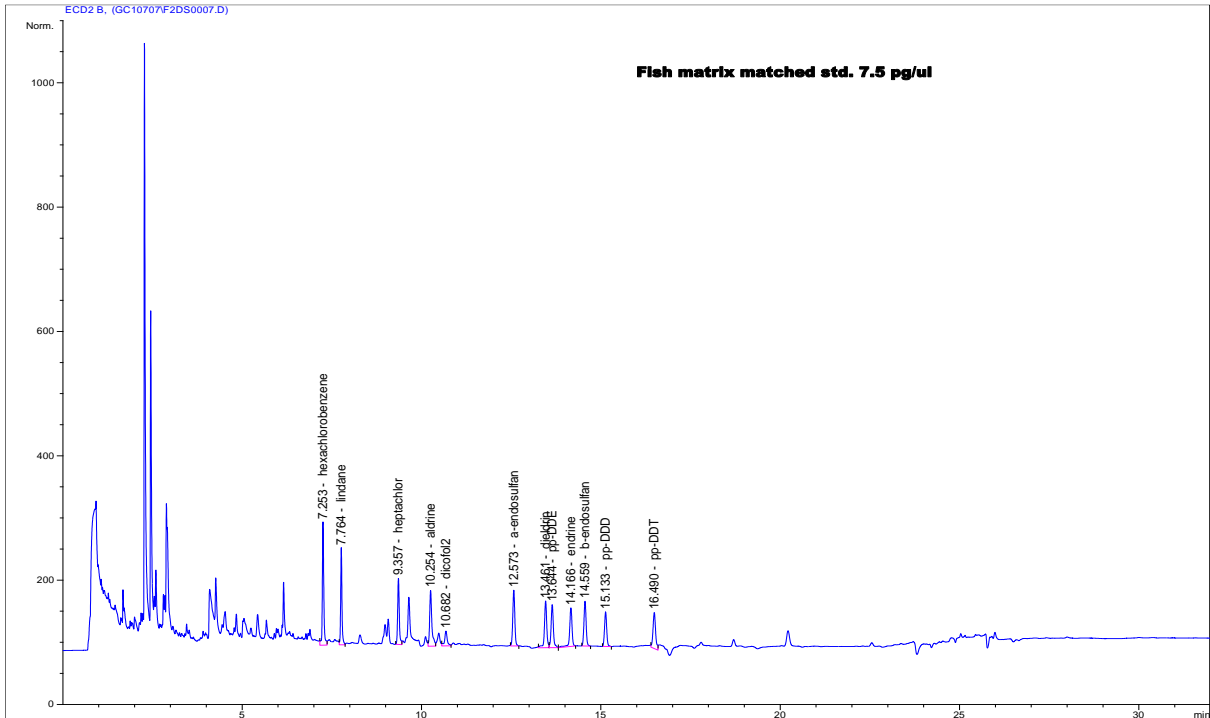
^c For intra-laboratory precision (repeatability)

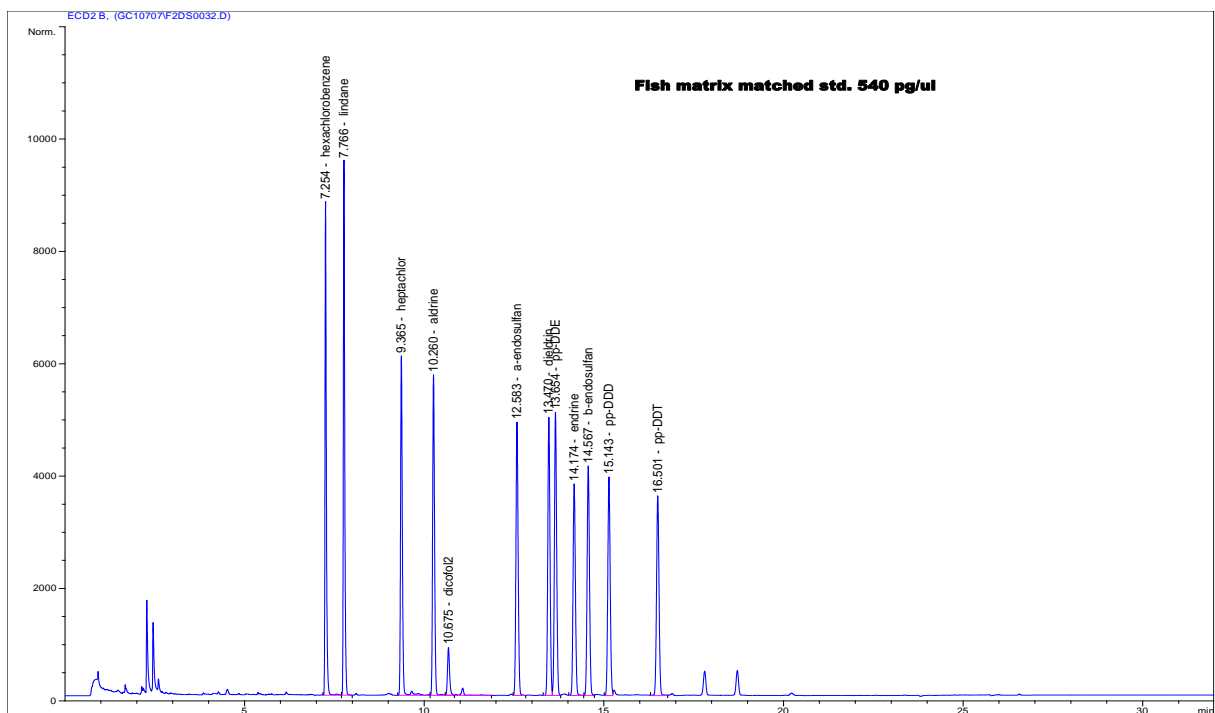
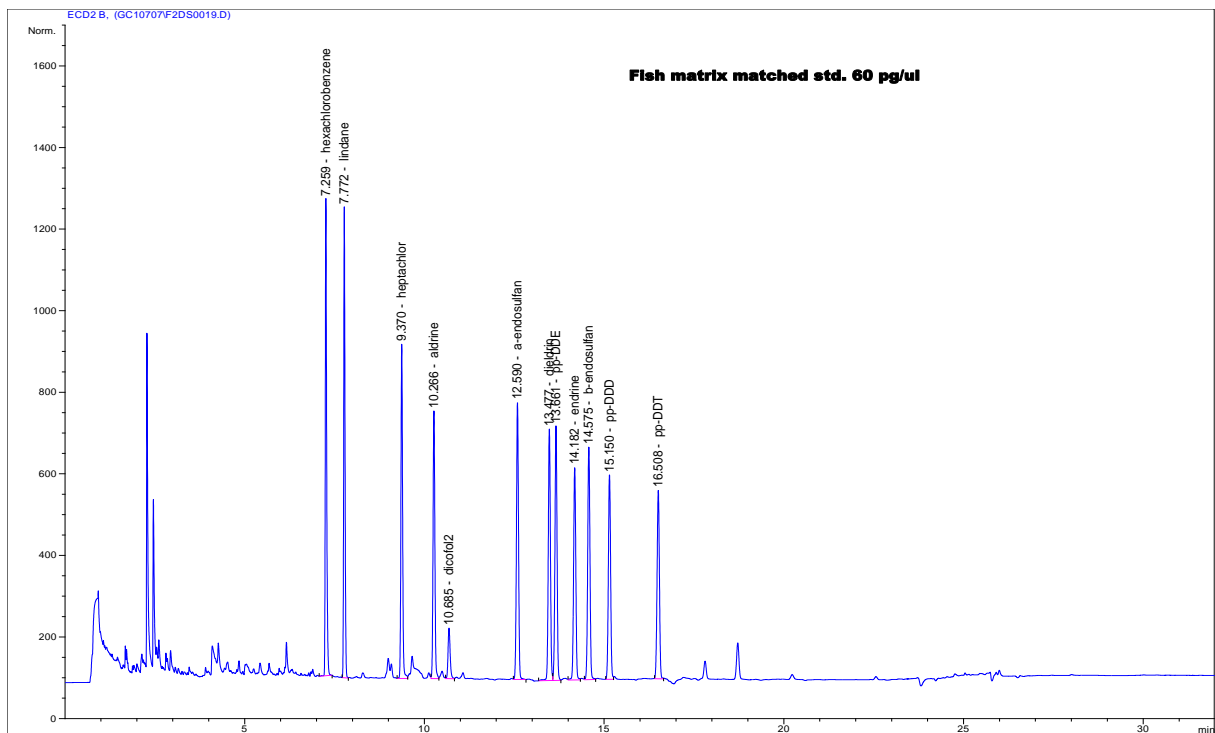
(A)



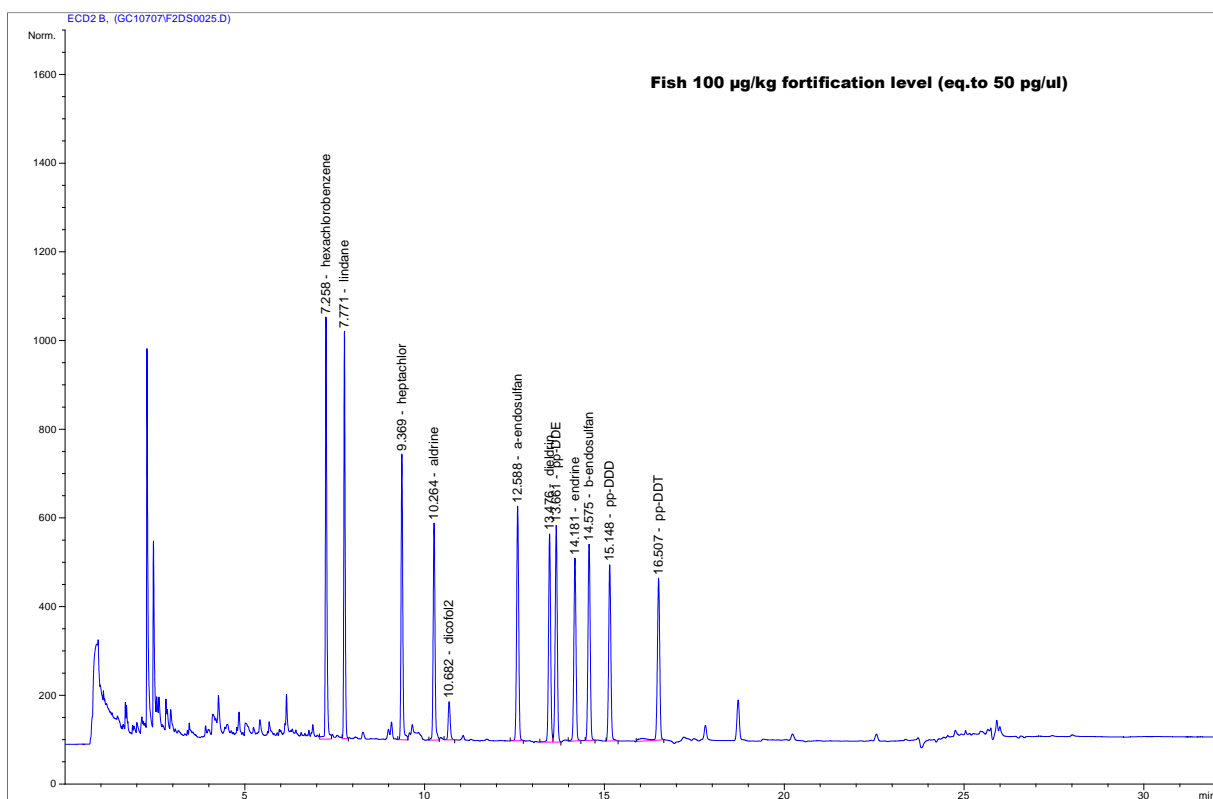
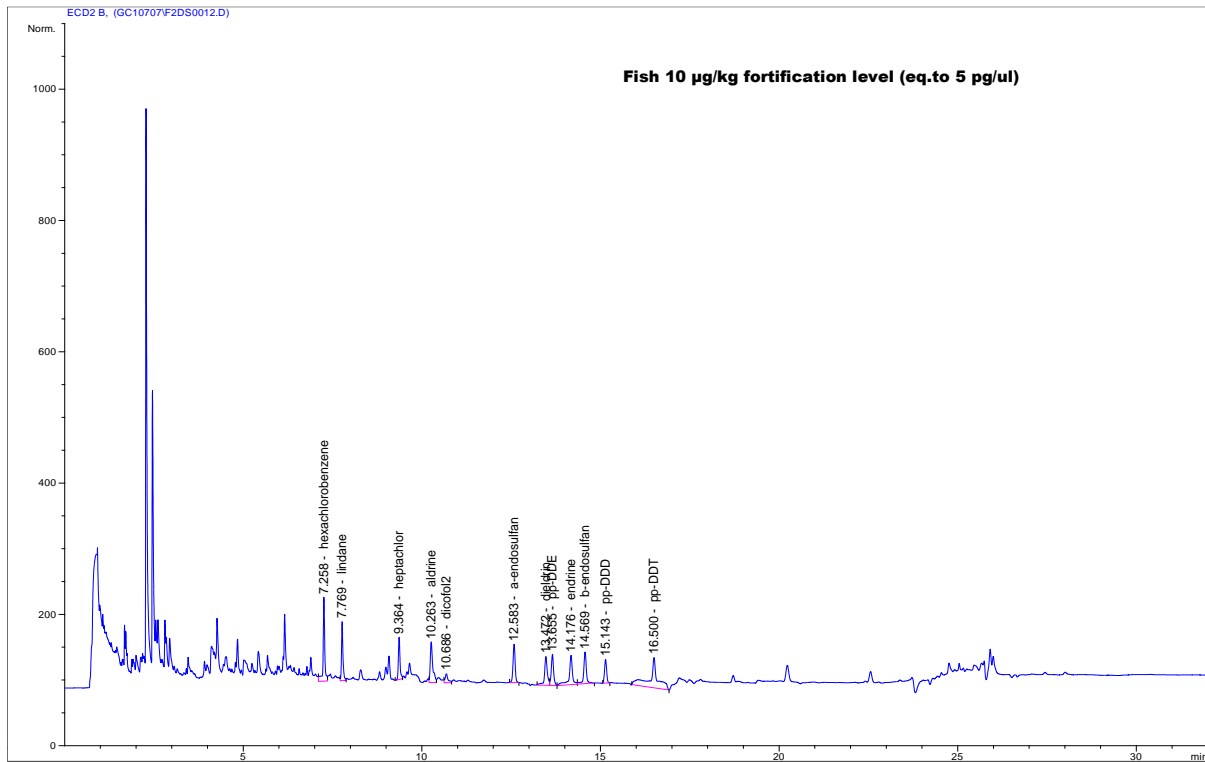


(B)





(C)



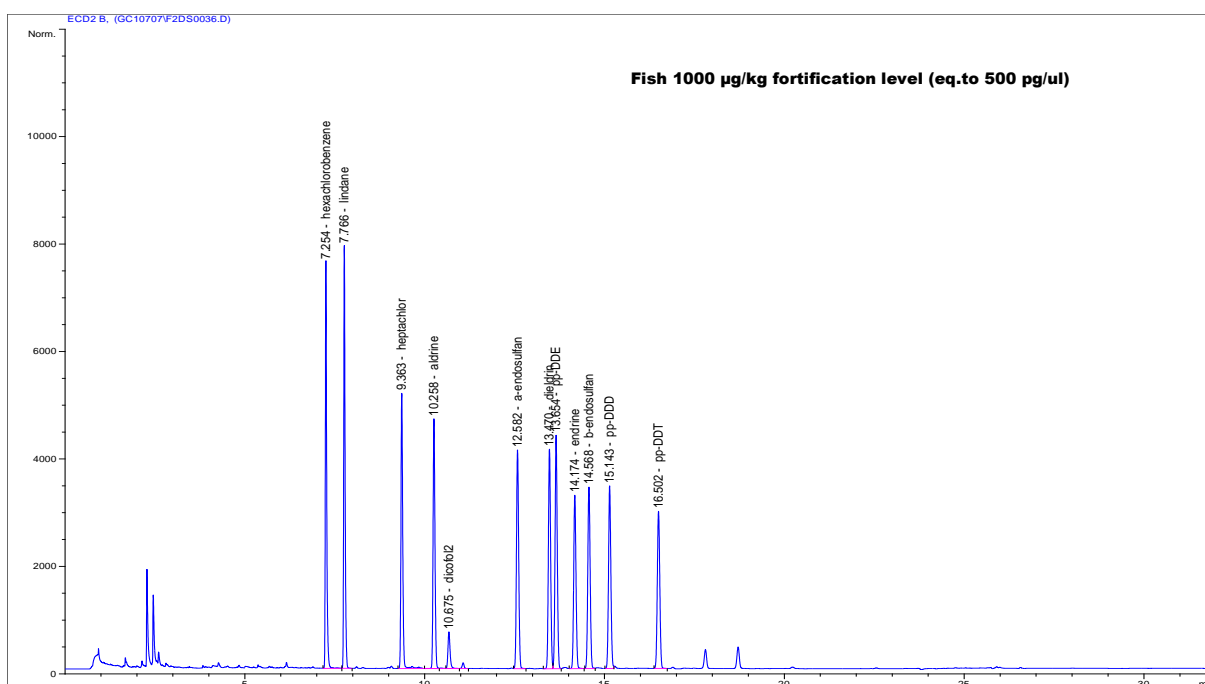


Figure 1. Representative GC-ECD chromatograms of pesticides for :
 (A) Reagent Blank and Fish blank
 (B) Fish matrix matched standards at 7.5 pg/ul, 60pg/ul and 540pg/ul
 (C) Fish fortified at 10 µg/kg, 100 µg/kg and 1000 µg/kg.

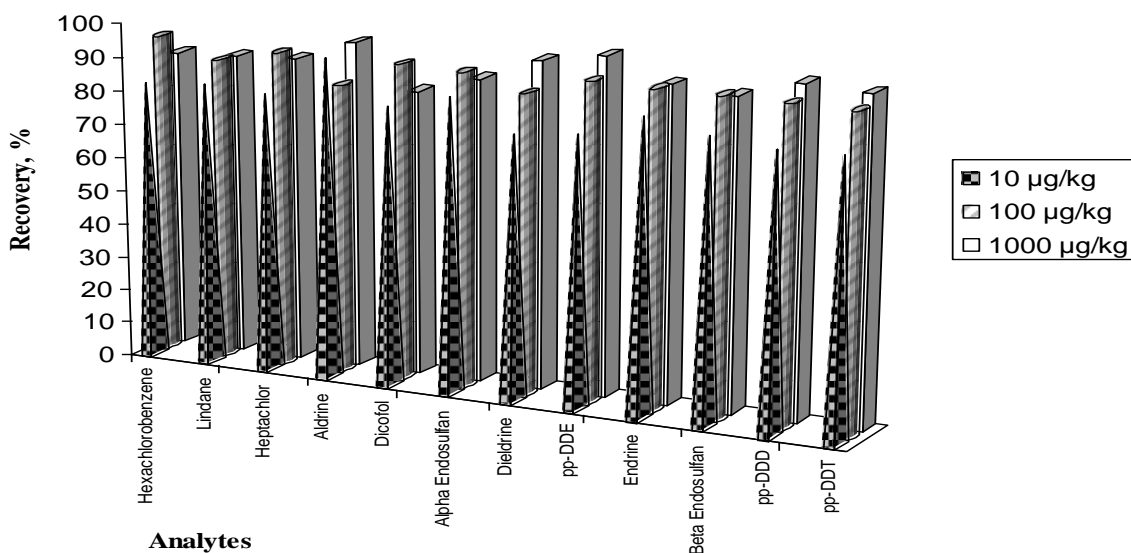


Figure 2. Recovery of the pesticides at three fortification levels (average of 2-occasions)