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Validation of Multi Residue Method for Organo Phosphate Pesticides in Water and Sediments by Gas Chromatography with Pulsated Flame Photometric Detector.

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ABSTRACT

Validation of multi residue method is a analytical technique for the determination of organo phosphate pesticides in water and sediments. The double distilled water is used as control for validation of multi residue method and sediments sample collected from the lake where the pesticide were not applied in catchment area. The investigated pesticides were selected on the based of such compounds that are commonly used organo phosphate pesticides around the world. The recoveries studies were performed at for water and sediments at different levels. The recoveries found to 96.5 % -87.5 % for 0.5 ppb level in water and 90% - 95.5 % in sediment. Based on the recovery results, the method mentioned above found to be genuinely suitable for the extraction and cleanup and hence the same method can be followed for the analysis of water and sediments for organophosphate pesticide on GC-PFPD.

Keywords: Validation of organo-phosphate pesticides, GC-PFPD , water and sediments

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INTRODUCTION

In modern agriculture practices farmers applying of large number of pesticide for protection crops from pests and diseases. The pesticide help to increase the production agriculture produce. Pesticides use is still indispensable in all countries in the area of agriculture also sanitary measures. Previous research reveals that the water and sediments were contaminated by pesticide. (Everaarts et al, 1997, Munga, 1985: Wandiga etal 2002). Therefore its was essential to know the contamination of environmental samples like water and sediments which pose human health hazards by the pesticides. The principal aim of this work was to develop a rapid multi residue method for the analyses of organo phosphate pesticides in water and sediments by modern detector Pulsated Flame Photometric Detector (PFPD) is which more sensitive than regularly used traditional detectors like Nitrogen Phosphorous Detector (NPD) and Flame Photometric Detector (FPD) which are used for orgno phosphate group pesticide analysis. The PFPD is have hydrogen rich flame is there inside, because of this the limit of detection of organs phosphate group pesticides higher than above detectors. An attempt was made to validated the analysis of organo phosphate group pesticides on the modified new Pulsated Flame Photometric Detector (PFPD). The most frequently used technique for analysis of pestidcide residues in water and sediments was Gas chromatography with different selective detectors as Flame photometric dector (Ueno etal 2004). The principal aim of this work was to develop a repid multiresidue method for analysis of organo phosphate pesticides in water and sediment samples on Gas Chromatogrtaphy with Pulsated Photometric Detector. The paper describes as simples and effective procedure for sample extraction, using a low volume of organic solvent and without cleanup particularly for sediments.

MATERIALS AND METHODS

For organo phosphate pesticides procedure validation before analysis the control water (glass double distilled water) and sediment samples were used for fortification recoveries studies. The method once again revalidated with standards organo phosphate pesticides viz., dichlorvos, monocrotophos, phorate, dimethoaate, phosphamidan, methyl parathion, fenitrothion, malathion, chlorpyriphos, chlorfenviphos, quinalphos, profenophos, ethion, triaazophos and phosalone on specific detector i.e., Pulsated Flame Photometric Detector . The Supelco pesticide standards with good purity was prepared in acetone, n- hexane. Pesticide stock solutions of 10 ppm was prepared and several standard individual pesticides and mixture of organo phosphate pesticide solution with concentrations 0.01 ,0.05, 0.1, 0.5, 1 ppm were injected to obtain the linearity of detector response and the detection limit of the pesticides, recoveries percentage , precision were studies for each pesticide on GC-PFPDwith coefficient (R^2) grater than 0.991. As per the Europen Union (1998) and BIS (2004) prescribed level of recoveries levels studied in water. The practically determined the LOQs by signal to Noise ration of 3 by spiking the ops mixture in water and sediments. The retention times of individual organo phosphate pesticides with respect to individual chromatograms and mixture chromatograme is giavaen Table 1. The method is suitable for the analysis of organo phosphate pesticides on PFPD in water and sediment/soil.

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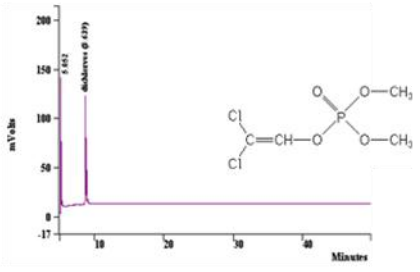
Standardizations were performed on Varian model CP-3800 (Netherlands) Gas Chromatography equipped with advanced Pulsated Flame Photometric Detector. A Varian factor four 1 ms, fused silica capillary column 30m x 0.25 m i.d., with 0.25 µm film thickness, supplied by Varian Technologies was employed. Operating conditions were as follow injector and detector temperature were 260^{0}C and 280^{0}C respectively and carrier gas and makeup gas both nitrogen was 1.5 ml and 25 ml respectively. The column temperature maintained with temperature programmed starting from 80^{0}C – 5 min – 20 ^{0}C – 150 ^{0}C - 5 min -5 ^{0}C -240 ^{0}C. The total analysis time for Organo phosphate pesticide was was 50 minutes. The volume of sample injected in split less mode was 1 µl. And the chromatograms of the individual organophosphate pesticide and mixture of the ops were shown in Fig.1 (all individual op pesticides) and Fig. 2 respectively. The identification and concentration of each compound was determined by comparing the peak retention time and peak areas in the fortified water and sediment sample with those found for mixture of pesticide of organo phosphate pesticide standards chromatogram.

Table 1

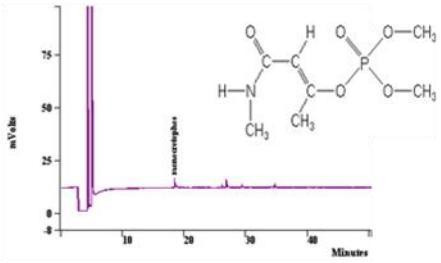
ORGANO PHOSPHATE PESTICIDES RETENTION TIMES ON PFPD			
Name of the Pesticide	Retention ime (Min)	Name of the Pesticide	Retention Time (Min)
Dichlorovas	8.653	Chlorpyriphos	26.900
Monochrotophos	18.56	Chlorfenviphos	29.273
Phorate	18.91	Quinalphos	29.460
Dimethoate	19.676	Profenophos	31.787
Phosphomedan	23.241	Ethion	34..200
Methyl prathion	23.782	Triazophos	34.787
Finitrothion	25.520	Phosalone	41.967
Malathion	26.235	-----	----

Standard One Nano Gram (1 ng) Organo Phosphates Chromatograms

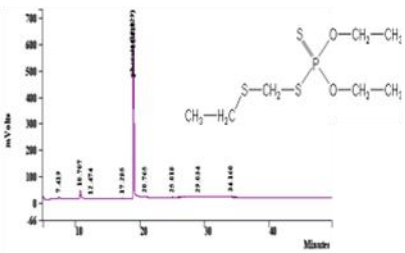
1. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate)



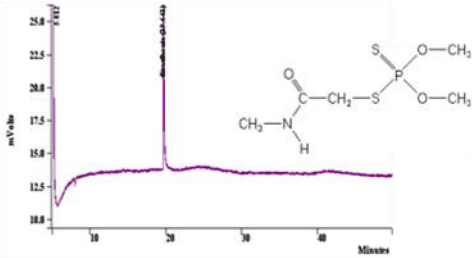
2. Monocrotophos (dimethyl (*E*)-1-methyl-2-ethyl carbamoyl)vinyl phosphate



3. Phorate (*O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate)



4. Dimethoate (*O,O*-dimethyl *S*-methyl carbanoylmethyl phosphorodithioate)



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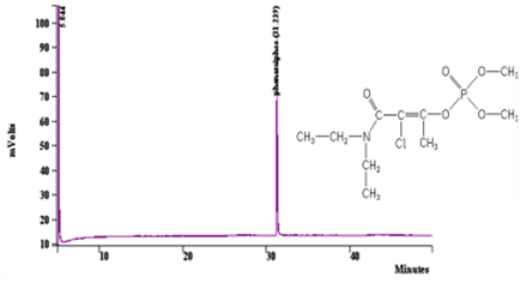
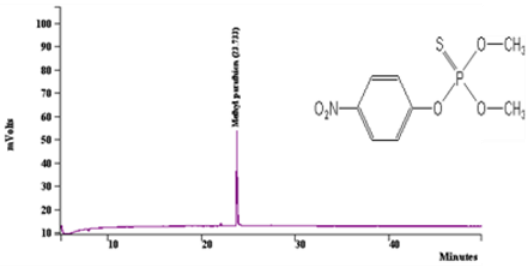
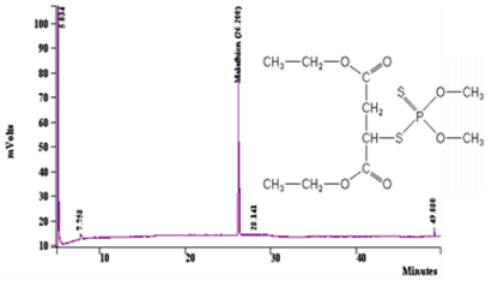
5. Phosphamidon (2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate



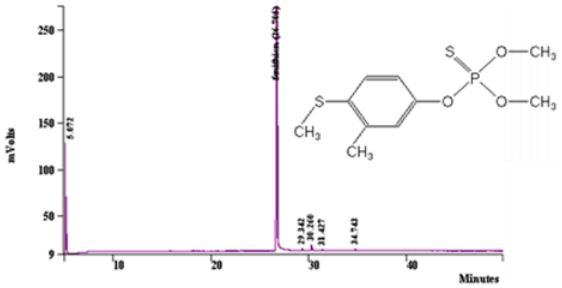
6. Parathion-methyl (O,O-dimethyl O-4-nitrophenyl phosphorothioate)



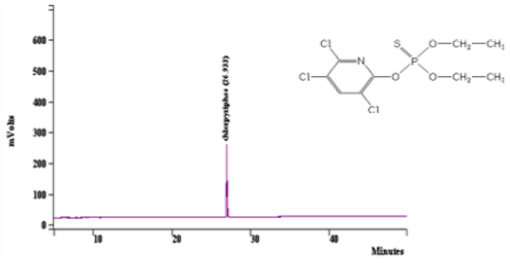
7. Malathion (diethyl (dimethoxy phosphinothioylthio)succinate)



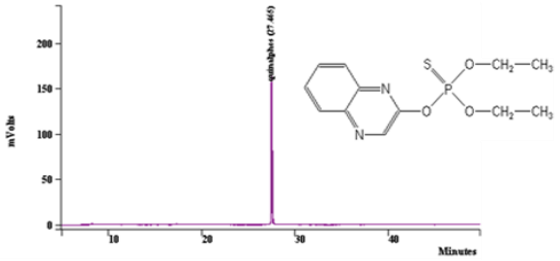
8. Fenthion (*O,O*-dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate)



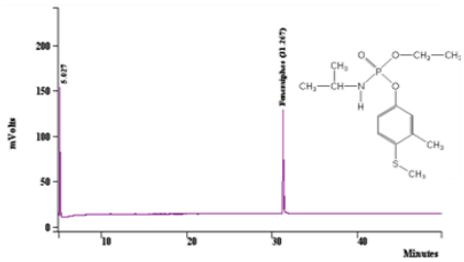
9. Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate)



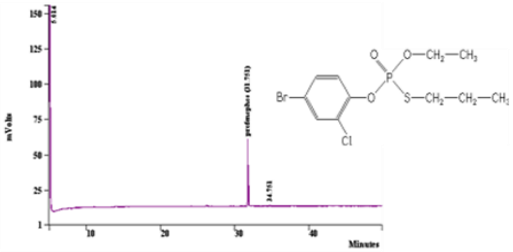
10. Quinalphos (O,O-diethyl O-quinoxalin-2-yl phosphorothioate)



11. Fenamiphos (ethyl 4-methylthio-*m*-tolyl isopropylphosphoramidate)



12. Profenofos (O-4-bromo-2-chlorohenyl O-ethyl *S*-propyl phosphorothioate)



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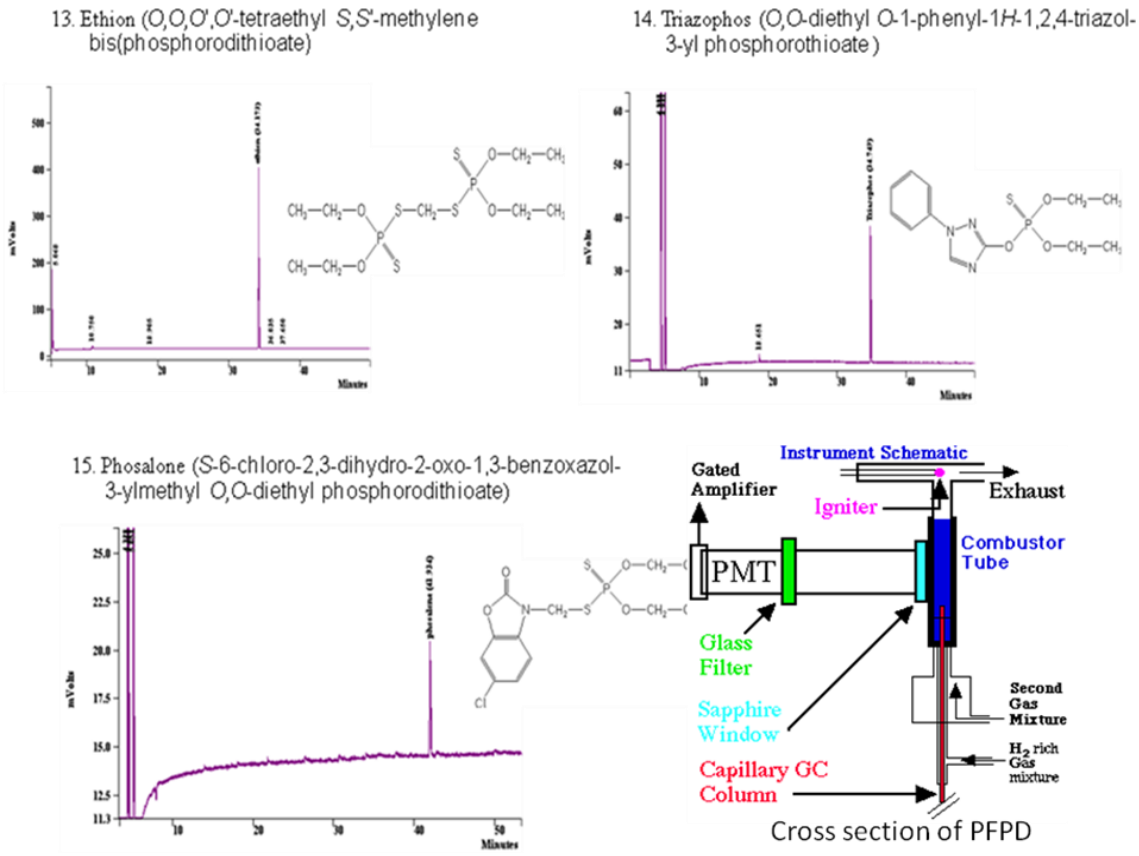

Figure 1 Standard One Nano Gram (1 ng) Organo Phosphates Pesticides Chromatograms and Cross Section of PFPD

Extraction cleanup of water

The glass doubled distilled water is taken as control for fortification recoveries studies. One liter of water sample is transferred into two liter capacity separatory funnel. To that the mixture of organo phosphate pesticide of 0.5 ml of 1 ppm (0.5 ppb level of fortification) is added. To that 100 g of pre-washed sodium chloride is added. The separatory funnel and the contents were vagarious shaken for 2-3 minutes till the sodium chloride salt is completely dissolved. Then 100 ml distilled dichloromethane (15% n-hexane in dichloromethane) was added and shaken vigorously releasing the pressure intermittently and kept undisturbed for separation of layers. After partitioning the lower organic layer (dichloromethane) was passed through anhydrous sodium sulphate supported on glass wool in funnel. The remaining aqueous layer was added with 50 ml of 15% n-Hexane in dichloromethane and partitioning was repeated twice collecting the lower organic layers. The three organic layers were pooled and concentrated on rotovap. The concentration process was repeated twice adding 10 ml hexane and each time to remove the traces of dichlormethane. The final volume of the extraction was made into 1 ml resulting in concentration factor of 1000 ml. From the final extract, 1 micro liter was injected in GC-PFPD, and the recoveries were calculated for individual pesticide in mixture of organo phosphates pesticide chromatogram. The same procedure followed for 0.1 ppb level and 0.01 ppb level recoveries. The chromatograms of recoveries were depicted in Fig. 2 to Fig. 4 .

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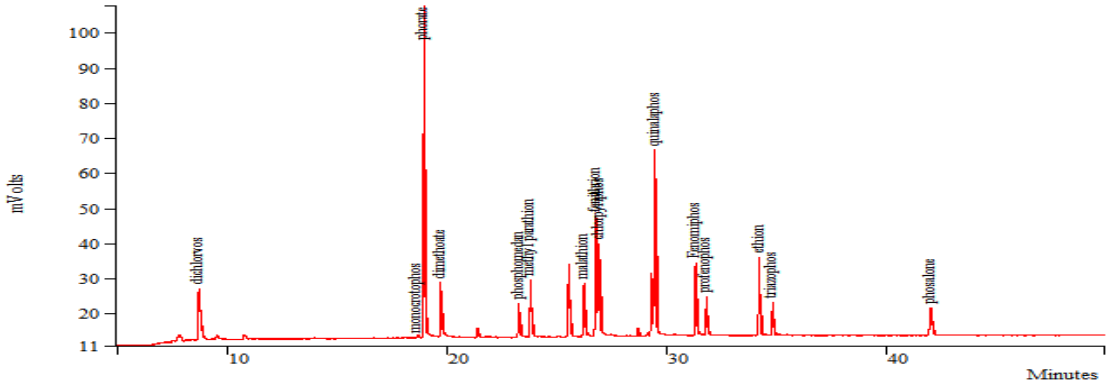

Figure 2: Chromatogram of standard mixture of organo phosphate pesticide 1 ng

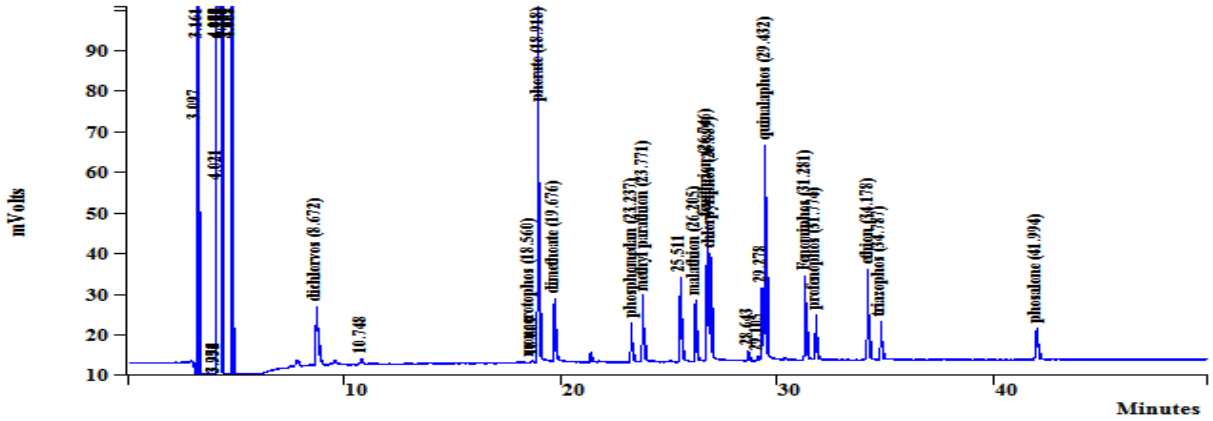

Figure 3 Fortification recovery chromatogram of ops mix at 0.5 ppb level in water

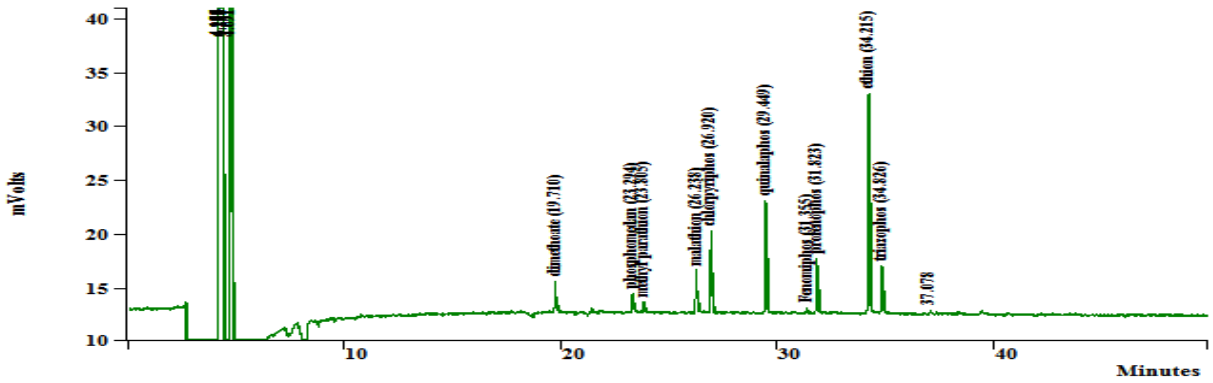

Figure 3 Fortification recovery chromatogram of ops mix at 0.1 ppb level in water

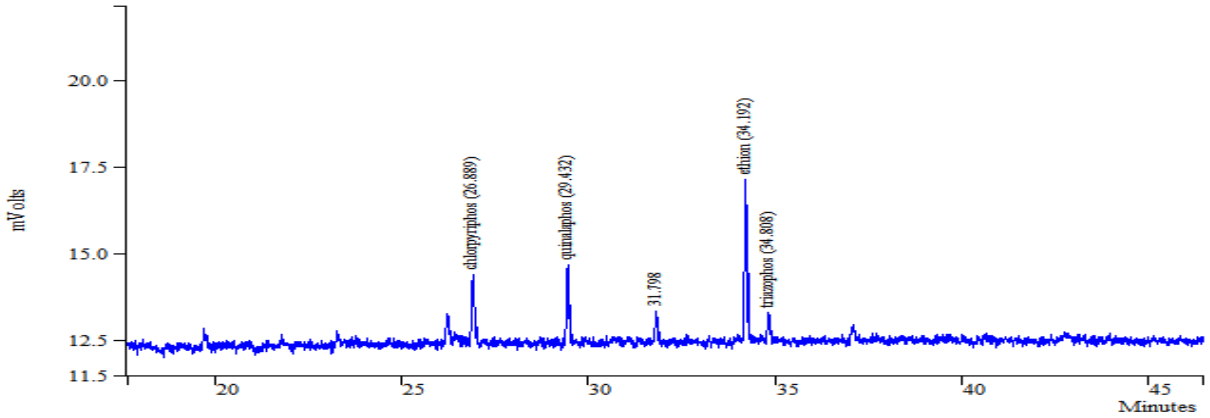

Figure 4: Fortification recovery chromatogram of ops mix at 0.01 ppb level in water

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Extraction and cleanup of soil

For the fortification recoveries of organo phosphate group pesticides in sediments, the shade dried sediment samples of 20 g of sewed sample was taken in beaker. To that 0.5 grams of floricil, 0.5 g of activated charcoal, 15 g of anhydrous sodium sulphate were thoroughly mixed with glass road. And 1ml of 0.2 ppm (1ppm level fortification) mixture of organo phosphate pesticides were added and again mixed thoroughly with glass road. All the contents were transferred to 60 mm x 25 mm column and eluted with 100 ml mixture n-hexane:acetone (9:1) mixture drop by drop for 5 hours. The elute is concentrated to dryness and dissolved in 5 ml n-hexane and anlysed on GC- PFPD with the samples GC parameter as in water analysis. The same procedure followed for other fortification recoveries concentrations i.e., 0.1 ppm level. The recoveries chromatograms were depicted in in Fig . 5 to Fig.6 .

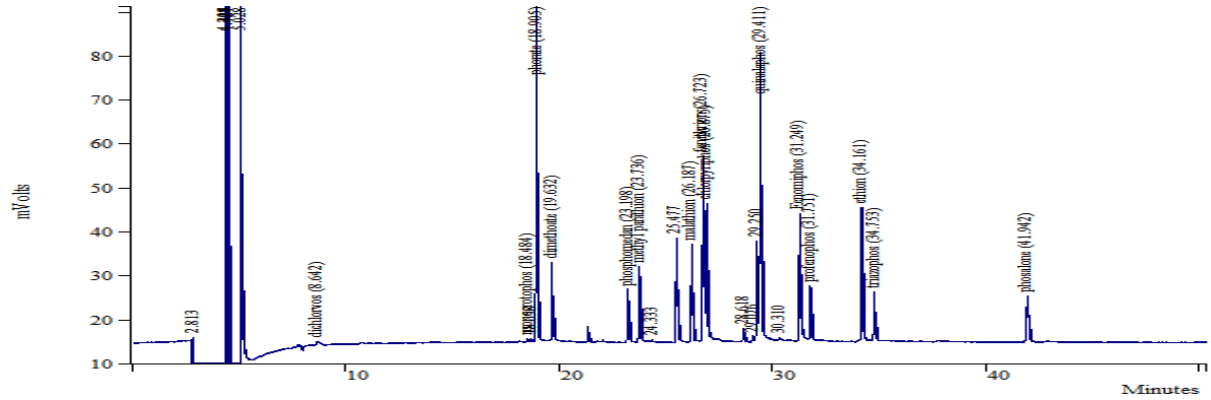

Figure 5 Fortification recovery chromatogram of ops mix at 1 ppm level in sediment

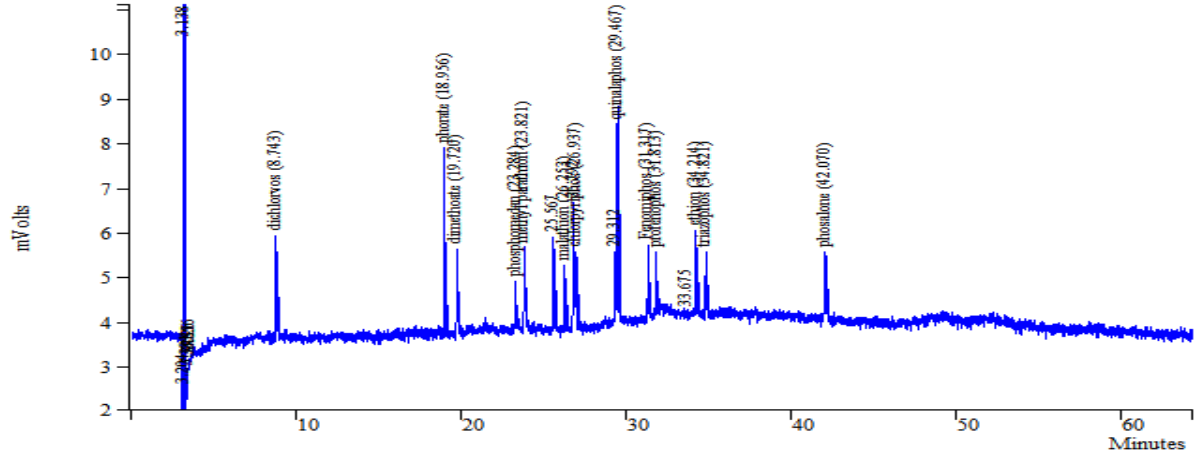

Figure 6 : Fortification recovery chromatogram of ops mix at 0.1 ppm level in sediment

RESULTS AND DISCUSSIONS

Method validation of organophosphate pesticide on PFPD in water

The spiked organo phosphate pesticides (ops) in water were studied for identification and quantification and the method is developed for analysis onGas Chromatography with Pulsated flame photometric detector (PFPD). The study was based on different organo phosphate pesticides standards at different fortification levels. The

identification of pesticides in multi residue chromatogram compared by observing retention times with individual ops standards retention times. The study of recovery for each pesticide in each matrix i.e., water and sediment at different concentration levels with respect to blank sample. And the percentage recoveries were calculated with respect to area obtained in the standard chromatogram and areas obtained in spiked chromatograms of water and sediment samples at different levels of concentrations.

The data presented in table.2 indicate that the percentage of recoveries organo phosphate pesticide obtained in water at 0.5 ppb , 0.1 ppb and 0.01 ppb levels. The recoveries of organo phosphates pesticide at 0.5 ppb in water is ranged 75% (monochrotophos) to 96.5% (chlorpyriphos). The recoveries of other organophosphate viz., dichlorvas 87.5%, phorate 92%, dimthoate 87.5% phosphomedan 92.5%, methyl parathion 94.5%, finitrothion 91.5%, malathion 95.0 %, chlorfenviphos 92%, quinalphos 93.5% , profenophos 95.%, ethion 95.0% triqazophos 92.0% and phosalone 91.5%. , while at 0.1 ppb level the recovery percentages were 0.00% to 87.0%. The recoveries were Nil recovered for monochrotophos, phorate, chlorfenviphos and phosalone and recoveries obtained for dichlorvas and dimethoate 80%, phosphomedan 87%, methyl parathion 90%, malathion 86%, chlorpyripos 91.5%, quinalphos 89%, profenophos 91.5%, ethion 93%, triazopos 75%. At the 0.01 ppb level the recoveries were very poor, out of 15 pesticides only 5 of the pesticides were recoveries ranged 65% to 82%. The only chlorpyriphos recovered in good quantity i.e., 82%. The remaining all ops recorded nil quantity of pesticides. The recoveries at 0.5 ppb level and 0.1 ppb level were good recoveries in relation to the recommended rate that range 70% - 120%. The results very similar with observation published by Huang et al (2010), N Reddy K et al (2010) and 2012), Tse H et al (2004). These two level are required for drinking water as per drinking water specifications European Union (1998). BIS (2004).

Table 2: Percentage Recoveries of Organo Phosphate Pesticides On GC - PFPD) In Water

Name of the Pesticide	% Recoveries at Various levels of Fortification					
	Level of Fortification (ppb) ug/L					
	0.5 ppb level	% Recovery	0.1 ppb level	% Recovery	0.01 ppb level	% Recovery
Dichorvas	0.875	87.5	0.80	80.0	0.00	0.00
Monochrotophos	0.75	72.0	0.00	0.00	0.00	0.00
Phorate	0.92	92.0	0.00	0.00	0.00	0.00
Dimethoate	0.875	87.5	0.80	80.0	0.00	0.00
Phosphomedan	0.925	92.5	0.87	87.0	0.00	0.00
Methyl prathion	0.945	94.5	0.90	90.0	0.00	0.00
Finitrothion	0.915	91.5	0.00	0.00	0.00	0.00
Malathion	0.95	95.0	0.86	86.0	0.00	0.00
Chlorpyriphos	0.965	96.5	0.915	91.5	0.82	82.0
Chlorfenviphos	0.92	92.0	0.00	0.00	0.00	0.00
Quinalphos	0.935	93.5	0.89	89.0	0.70	70.0
Profenophos	0.95	95.0	0.915	91.5	0.605	60.5
Ethion	0.95	95.0	0.93	93.0	0.70	70.0
Triazophos	0.92	92.0	0.75	75.0	0.65	65.0
Phosalone	0.915	91.5	0.00	0.00	0.00	0.00

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Method validation of organophosphate pesticide on PFPD in sediments

The sediment samples spiked with organo phosphate pesticides were studied and method is developed for identification and quantification. The study was based on different ops standards at different fortified levels. The various levels of fortification i.e., 1 ppm and 0.1 ppm were investigated. The chromatograms obtained were compared with standard chromatograms of the same levels for identification and quantification.

The perusal of data indicated in Table.3 reveled that the percentage recoveries of organo phosphate pesticide obtain in sediments at 1 ppm and 0.1 ppm levels. The recoveries of organo phosphate pesticices at 1 ppm and 0.1 ppm levels recorded ranged 50% to 95.5% and at 0.1 ppm level Nil – 90%. The recoveries of individual pesticides viz., dichlorvas 70% - 92%), phorate (0.00 - 92%), monocrophos (Nil – 50%), dimethoate (0.00 – 93%), phosphomedan (80% - 94.5%), methyl parathion 90% - 97%), finitrothion (50% - 89%), malathion (67% - 90%), chlorpyriphos (89.5% - 95.5%), chlorfenviphos (65% to 94%), quinalphos (Nil – 90.5%), profenophos (81.5% - 94.5%), ethion (74% - 93%), triazophos (56% - 89%) and phosalone (77% - 90.5%) respectively. The findings of are very close to the results of Seema Tahir (1999), M.Bujagendra Raju (2011) and M.I.Wabel (2010).

Table 3: Percentage Recoveries of Organo Phosphate Pesticides on GC - PFPD in Sediments

Name of the Pesticide	1 ppm level	% Recovery	0.1 Ppm level	% Recovery
Dichorvas	0.92	92.0	0.70	70.0
Phorate	0.92	92.0	0.00	0.00
Monocrophos	0.50	50.0	0.00	0.00
Dimethoate	0.93	93.0	0.00	0.00
Phosphomedan	0.945	94.5	0.80	80.0
Methyl parathion	0.97	97.0	0.90	90.0
Finitrothion	0.89	89.0	0.50	50.0
Malathion	0.90	90.0	0.67	67.0
Chlorpyriphos	0.955	95.5	0.895	89.5
Chlorfenviphos	0.94	94.0	0.65	65.0
Quinalphos	0.905	90.5	0.00	0.00
Profenophos	0.945	94.5	0.815	81.5
Ethion	0.93	93.0	0.74	74.0
Triazophos	0.89	89.0	0.56	56.0
Phosalone	0.905	90.5	0.77	77.0

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