

METHOD VALIDATION FOR DETERMINATION OF 23 ORGANOPHOSPHORUS PESTICIDE RESIDUES IN CHILLI BY GAS CHROMATOGRAPHY

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ABSTRACT

Laboratory method was validated for the determination of 23 organophosphorus pesticides in chilli. QuEChERS extraction method using ethylacetate as extracting solvent followed by use of sodium sulphate and magnesium sulphate for moisture removal. Cleanup of samples was done using PSA, C18 and Activated charcoal. LOD (Limit of Detection) and LOQ (Limit of Quantification) of the pesticide for chilli was calculated. LOD value is lowest for phorate (0.005 mg/kg) and highest for parathion methyl (0.1 mg/kg) whereas LOQ value is lowest for phorate (0.015 mg/kg) and highest for parathion methyl (0.3 mg/kg). MRL (Maximum Residue Limit) values

of phorate is lowest (0.05mg/kg) and highest for malathion (3.0 mg/kg) whereas ADI (Acceptable Daily Intake) values for diazenon, malathion, phasalone are highest (0.02 mg/kg) and lowest for fenthion, monocrotophos (0.0005mg/kg). Recovery studies at 0.05 ppm level of standard mixture of 23 pesticides was carried out and was found that recovery lies within the range of 82.67% to 93.33%. It is observed that mean recovered amount at 0.05 mg/kg spike concentration of 23 pesticides in chilli matrix are in the range of 0.0413 mg/kg to 0.0467 mg/kg and highest recovery percent for chlorpyrifos (93.33%) and lowest recovery percent for ethion (82.67%), rest pesticides recovery percent in between the range whereas relative standard deviation are in the range of 2.2 to 8.571, these values lies below codex permissible limit of 15. Linearity was checked by calibration curve, plotted between an area

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of 23 pesticide standard mixture against seven different concentration levels of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/kg with regression co-efficient (R^2) are in the range of 0.978-0.994. Edifenphos with lowest (R^2) 0.978 and Monocrotophos with highest (R^2) 0.9974, rest all pesticides coefficient of regression lies between these value.

KEYWORDS: Chilli, Pesticides, LOD, LOQ, MRL, QuEChERS.

INTRODUCTION

The use of pesticides provides unquestionable benefits in increasing agricultural production. The establishment of legal directives to control their levels through the Maximum Residue Levels (MRLs) and on the other, a continuous look for pesticides which are less persistent and toxic to humans. increased tremendously the number of pesticides registered and recommended the analytical difficulties for their control.^[1] Analytical methods are needed to screen, quantify and confirm the pesticide residues in fruits and vegetables for both research and regulatory purpose. Multiresidue methods (MRMs) and single residue methods (SRMs) generally consist of the same basic steps, but MRMs are preferred to the latter for the analysis of pesticides, because MRMs provide the capability of determining different pesticide residues in a single analysis run. A review of the existing methods used to extract, isolate, and quantify pesticide residues in fruits and vegetables by monitoring agencies, demonstrates that they are based on classical MRMs, some developed over 30 years ago.^[1] Among the more widely used MRMs are those of Mills; Mills, 93 Onley and Gaither; Storherr; Luke; and Krause.^[2]

The method adopted by the Association of Official Analytical Chemists (AOAC) is the internationally recognized procedure for MRM. It allows the determination of many pesticide residues in vegetables and involves an aqueous acetone extraction but with laborious cleanup. Such methods, generally, involve an extraction step with a water miscible solvent, followed by a cleanup step, with an organic solvent of limited water capacity, to achieve the removal of interferences present in the sample extract and solid phase cleanup with silica or florisil. Finally the analyte determination is performed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) with selective detectors.^[1] The permissible levels of pesticide residues in food are controlled by the MRLs, which are established by each country and may cause conflicts because the maximum residue levels acceptable in a particular one country may be unacceptable in another. To overcome this problem, there is a need to harmonize the different MRLs, adopted by different countries and this has been

addressed by two international organizations: the European Union (EU) at European level and the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).^[1] However, in the recent past, improvements in the sample preparation techniques for different environmental samples have led to modification of the existing methods and development of new techniques, in order to save time and reduce use of chemicals and thus improve the overall performance of analytical process. As a result, several rapid, low cost, environment friendly and readily automated methods of extraction are now available. Besides, because of the complexity of the matrices, extraction is usually followed by very specific clean-up procedures to achieve accurate sample quantification, so the new methods are modified in order to achieve a compromise between cost, selectivity and sensitivity. Future developments in all areas of analytical sample preparation are expected to continue to be application-driven in a quest for improved recovery, higher sample throughput and reduced consumption of organic solvent with capability to provide accurate results.^[3] As a result, modern sample preparation procedures, such as accelerated solvent extraction (ASE)^[4], supercritical fluid extraction (SFE)^[5], microwave assisted extraction (MAE),^[6] solid phase extraction (SPE)^[7], solid phase micro extraction (SPME),^[8] matrix solid phase dispersion (MSPD),^[9] extraction and QuEChERS (quick, easy, cheap, effective, rugged and safe),^[10] have been developed to overcome the drawbacks of the traditional approaches. Contamination of vegetables result from pesticide spray, as well as from improper handling, contaminated environment (air, soil or water) and from cross contamination processes.^[11] India is one of the few remaining countries still engaged in the large scale manufacture, use and export of some of the toxic chlorinated pesticides, such as p, p'-dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH) and pentachlorophenol (PCP).^[12]

MATERIAL AND METHODS

Chemicals and Reagents

Pesticide reference standards (Sigma-Aldrich), Ethyl Acetate (HPLC grade), n-Hexane (HPLC grade), anhy. Na₂SO₄ (Merck), anhy. MgSO₄ (Merck), Primary Secondary Amine (PSA), (Agilent Technologies), and graphitized carbon black sorbent Supelco (Bellefonte, PA, USA).

Extraction

Chilli was purchased from local vegetable mandi of Satna District. Purchased sample was transported in ice pack to the laboratory in cool-box, stored at 4°C and extracting within 48 h. QuEChERS (quick, easy, cheap, effective, rugged and safe) method^[13] was followed for extraction of pesticides from chilli samples. Chilli samples were chopped and homogenized. Fifteen gram of homogenized sample weighed into centrifuge tube and ethyl acetate (30 ml) was added and shaken for 1 min. Ten gram anhydrous Na₂SO₄ was added and shaken for 30 min by rotospin and were centrifuged at about 5 minutes. Cleaning up was done according to Lehotay (2007).^[14] 6 ml extract was transferred from the upper layer into a 15 ml tube and 0.9 g anhydrous MgSO₄, 0.25 g PSA and 0.25 g activated charcoal were added and shaken for 1 min. The tubes were centrifuged and supernatant 4 ml was dried and finally make up to 1 ml for injection in GC-FPD (Gas Chromatography-Flame Photometric Detector) and GC-MS (Gas Chromatographic-Mass Spectrometer).

Instrument Conditions

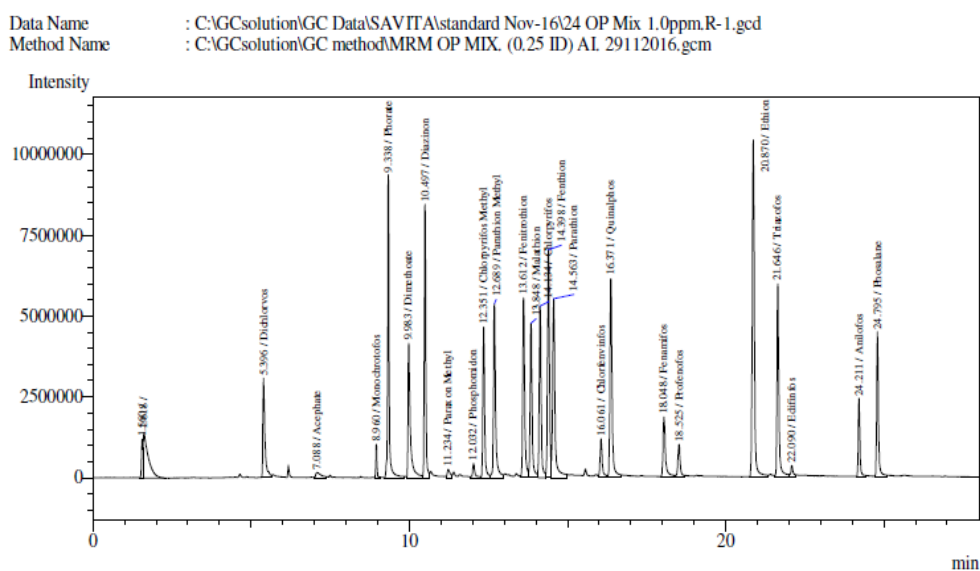
Shimadzu make GC-FPD (GC-QP 2010 model) with AOC-20S Auto Sampler equipped with DB-5MS fused silica capillary column (Agilent J&W GC column, 5% Phenylated methyl siloxane, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness) was used for preliminary screening and final quantification of pesticide residues. Analysis was performed with oven temperature programming of 100°C as initial temperature for 2 min followed by a ramp rate of 25°C/min up to 200°C for 5 min., 4°C/min upto 230°C for 2 min and 20°C/min up to final temperature of 280°C with a hold time of 5 min. The injector and detector temperature was set at 250°C, 290°C, respectively. Sampling rate 40 msec, injection volume 1.0 micro litre with high plunger speed and 0.5 min equilibrium time. The instrument was set in split mode of (10:1). Helium was used as makeup gas and also as carrier gas at a flow rate of 1.23 mL/min. H₂ flow rate 85ml/min with air flow 110.0 ml/min.

GC Model 7890A (Agilent Technologies) with mass (5975C inert XL EI/CI MSD) triple axis detector was used for pesticide residue analysis. Injector port temperature was set at 280°C. DB-5MS fused silica capillary column (Agilent J&W GC column, 5% Phenylated methyl siloxane, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness) was used with linear flow at 1ml/min. 1 micro litre of concentrated extract was injected in splitless mode with carrier gas helium (grade-1). The pesticides were separated with 40.75 min oven programming of initial temperature 50 °C for 2 min followed by a ramp rate of 8°C /min up to a temperature of 280°C

with a hold time of 10 min. The injector was operated in splitless mode at 280°C temperature. The interface, ion source and quadrupole temperatures were set at 280°C and 230°C and 150°C respectively. The mass spectrometer was operated in electron impact (70eV), selected ion monitoring (SIM) and Scan mode with solvent delay time 3 min. The scanning mode offers enhanced selectivity over either full scan or selected ion monitoring (SIM).

Standard Stock Preparation

Pesticide taken for the study, their stock solution of was prepared by Certified Reference Materials (CRM) of pesticide with specific purity mentioned. Clean and dried standard volumetric flask of 10 ml was taken. CRM of individual pesticide weighed directly on analytical balance pan (Mettler, Toledo) maximum up to 2mg, dissolved in few drops of HPLC grade acetone which is further make up to the mark of standard volumetric flask with HPLC grade hexane. Standard stock solution and working standards were stored in deep freezer at -20°C. 23 Organophosphorus commonly used viz Anilophos, Chlorfenvinfos, Chlorpyrifos, Chlorpyrifos-methyl, Dichlorvos, Ethion, Malathion, Parathion methyl, Monocrotophos, Phorate, Profenofos, Quinalphos, Trizophos, Fenitrothion, Phosalone, Paraxon-methyl, Fenamiphos, Edfinphos, Dimetoate, Diazinon, Fenthion, Parathion and Phosphomidon, standard solution mixture was prepared at seven different concentration levels of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/kg, All pesticides taken for study gave good response for FPD detector. All these working standard solutions of a mixture of pesticides were prepared for calibration and recovery tests.



RESULT AND DISCUSSION

Method validation and residue screening of chilli samples were conducted by GC-FPD for the presence of pesticides whereas concentrated samples were analysed by GC-MS in full scan mode for further confirmation of pesticides. Presence of pesticides in samples was confirmed with the help of two parameters, namely Retention Time (RT) and Mass Spectrum (MS). Matching of RT and MS data of the sample peak with that of the standard confirms identification of the pesticides present in the sample. Final quantification was carried out on GC-FPD. In-house validation of the method was carried out on chilli matrix for 23 organophosphorus pesticides.

Pesticides taken for study

23 Organophosphorus pesticides which are commonly used in India viz Anilophos, Chlorfenvinfos, Chlorpyrifos, Chlorpyrifos-methyl, Dichlorvos, Ethion, Malathion, Parathion methyl, Monocrotophos, Phorate, Profenofos, Quinolpos, Trizophos, Fenitrothion, Phosalone, Paraxon-methyl, Fenamiphos, Edfinphos, Dimetoate, Diazinon, Fenthion, Parathion and Phosphomidon are considered for study. Preliminary screening was done to find out the best chromatographic techniques can be used in terms of peak shape, response and LOD/LOQ. All 23 pesticides gave good response for FPD detector which can be used for quantification and confirmation by GC-Mass Spectroscopy.

Method Development and Validation

Validation is the test for the suitability of a particular method for collecting analytical data. Parameters for ensuring a robust method, they are - Recovery, Selectivity (Specificity), Calibration, Repeatability, Reproducibility, Limit of Detection (LOD), Limit of Quantitation (LOQ), (European Commission, Directorate General Health and Consumer Protection, SANCO/2007/3131(Supersedes Document No. SANCO/10232/2006).

Limit of Detection and Limit of Quantification

LOD & LOQ will be measured by using EPA method as it is simple, easy and practical to implement.^[15,16] Limit of Detection (LOD) and Limit of Quantification (LOQ) was calculated as the lowest concentration a pesticide in a selected matrix which gave signal to noise (S/N) ratio of approximately equals to 3 and 10 respectively. To measure the LOD, peak to peak noise of blank matrix (chilli) at or around the retention time of individual pesticides, chromatogram of standard mixture is noted and averaged for three replicates. Concentration of the individual pesticide is calculated (in µg/g) from the matrix spiked chromatogram which

could produce the signal equal to three times of blank matrix. LOQ is calculated by multiplying the LOD value by factor 3 round of two decimal place value. Measured method LOD, LOQ and the relevant legislative limits (MRLs) and ADI.^[17] are listed in table 1. With the present method, all 23 organophosphorus pesticides for study presented LOD and LOQ were in the range of below MRL. From table-1, it was observed that for chilli matrix, LOD value is lowest for phorate (0.005 mg/kg) and highest for parathion methyl (0.1 mg/kg) whereas LOQ value is lowest for phorate (0.015 mg/kg) and highest for parathion methyl (0.3 mg/kg). MRL values of phorate is lowest (0.05mg/kg) and highest for malathion (3.0 mg/kg) whereas ADI values for diazenon, malathion, phasalone are highest (0.02 mg/kg) and lowest for fenthion, monocrotophos (0.0005mg/kg). MRL values for some pesticides like chlorpyrifos methyl, quinolphos, fenamiphos, profenophos, trizophos, edfinphos, anilophos are not available whereas ADI values are not available for monocrotophos, paraxon-methyl, parathion, chlorfenvinphos, quinolphos, fenamiphos, profenophos, ethion, trizophos edifenphos and anilophos.

Table-1. Shows Class, Retention Time (R.T), limit of detection (LOD), limit of quantification (LOQ), maximum residues limits (MRL) and acceptable daily intake (ADI) of Organophosphorous pesticide spiked in chilli matrix by GC-FPD.

S.No.	Organophosphorous Pesticides	R.T	LOD (mg/kg)	LOQ (mg/kg)	MRL (mg/kg)	ADI (mg/kg/day)
1	Dichlorvos	5.49	0.007	0.021	0.15	0.004
2	Monocrotophos	9.16	0.03	0.09	0.2	NA
3	Phorate	9.44	0.005	0.015	0.05	0.0005
4	Dimetoate	9.86	0.04	0.12	2	0.002
5	Diazinon	10.43	0.06	0.18	0.50	0.02
6	Paraxon-methyl	11.22	0.05	0.15	0.2	NA
7	Phosphomidon	12.03	0.05	0.15	0.2	0.004
8	Chlorpyrifos-methyl	12.44	0.02	0.06	NA	0.01
9	Parathion methyl	12.67	0.1	0.3	1.00	0.003
10	Fenitrothion	13.61	0.03	0.09	0.30	0.005
11	Malathion	13.88	0.01	0.03	3.00	0.02
12	Chlorpyrifos	14.25	0.02	0.06	0.20	0.01
13	Fenthion	14.25	0.03	0.09	1	0.0005
14	Parathion	14.43	0.01	0.03	0.5	NA
15	Chlorfenvinfos	15.95	0.04	0.12	0.5	NA
16	Quinolphos	16.42	0.01	0.03	NA	NA
17	Fenamiphos	17.86	0.02	0.06	NA	NA
18	Profenofos	18.58	0.03	0.09	NA	NA
19	Ethion	20.93	0.01	0.03	1.00	NA
20	Trizophos	21.60	0.02	0.06	NA	NA
21	Edfinphos	21.99	0.01	0.03	NA	NA

22	Anilophos	24.15	0.05	0.15	NA	NA
23	Phosalone	25.74	0.02	0.06	1	0.02

Linearity

The calibration curve of GC-FPD instrument are created at seven levels i.e (matrix match) prepared by spiking 23 pesticides standard mixture solution at different concentration levels and injected in triplicate. Figure 1 & 2 shows calibration curve, plotted between an area of 23 pesticide standard mixture against seven different concentration levels of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/kg with regression co-efficient (R^2) are in the range of 0.978-0.994. Edifenphos with lowest (R^2) 0.978 and Monocrotophos with highest (R^2) 0.9974, rest all pesticides coefficient of regression lies between these value. Figure 1 & 2 shows calibration curve of 23 organophosphorus pesticides mixture of concentration 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 mg/kg in GC-FPD with coefficient of regression (R^2).

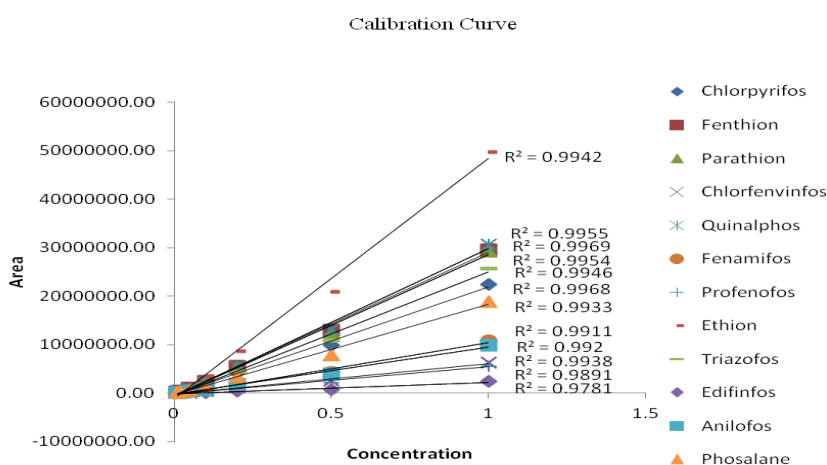


Fig-1. Calibration curve of OP mix. at conc 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 mg/kg.

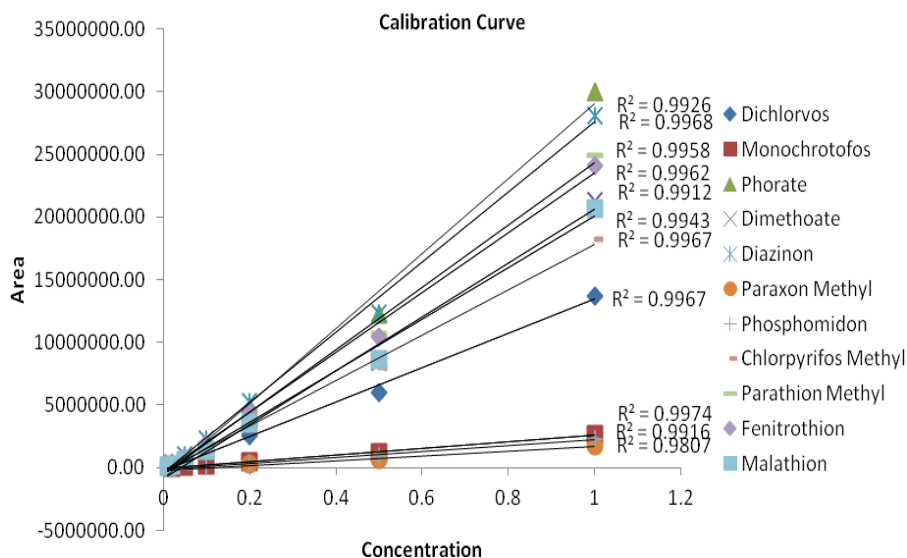


Fig-2. Calibration curve of OP mix. at conc 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 mg/kg.

Recovery

Method trueness was assessed by recovery studies using chilli matrix spiked at 0.05 ppm concentration level and injected in three individually prepared replicates. Spiking of samples occurred prior to sample preparation, recovery, standard deviation and relative standard deviation (% RSD) are calculated. Table 2 shows recovery, standard deviation and relative standard deviation for organophosphorus pesticides. From table 1, it is observed that mean recovered amount at 0.05 mg/kg spike concentration of 23 pesticides mixture of organophosphorus in chilli matrix are in the range of 0.0413 mg/kg to 0.0467 mg/kg and it is observed that highest recovery percent is for chlorpyrifos (92.67%) and lowest recovery percent is for ethion (82.67%) rest pesticides recovery percent in between the range whereas relative standard deviation are in the range of 2.2 to 8.571 values lies below codex permissible limit of 15. According to SANCO, recovery values should come within acceptable range between 70–120%. It always happens that matrix effect was predominant for some pesticides. To overcome variable matrix effect, the quantification was done using matrix-matched standards prepared in matrix blank.

Table-2. Shows Recovery, Mean Recovery, Standard Deviation (S.D), Relative Standard Deviation (RSD) of Organophosphorus pesticides from spiked chilli matrix at 0.05 ppm.

S.NO.	PESTICIDE	RT	Spiking conc	R1		R2		R3		Mean Rec. Amount	Mean recovery	S.D.	R.S.D
				Amount recovered	Recovered percent	Amount recovered	Recovered percent	Amount recovered	Recovered percent				
1.	Dichlorvos	5.49	0.05	0.043	86	0.045	90	0.04	80	0.0427	85.33	0.0025	5.859
2.	Monocrotophos	9.16	0.05	0.043	86	0.044	88	0.045	90	0.0440	88.00	0.001	2.273
3.	Phorate	9.44	0.05	0.045	90	0.042	84	0.043	86	0.0433	86.67	0.0015	3.462
4.	Dimetoate	9.86	0.05	0.047	94	0.046	92	0.043	86	0.0453	90.67	0.0021	4.632
5.	Diazinon	10.43	0.05	0.043	86	0.048	96	0.046	92	0.0457	91.33	0.0025	5.474
6.	Paraxon-methyl	11.22	0.05	0.043	86	0.04	80	0.043	86	0.0420	84.00	0.0017	4.048
7.	Phosphomidon	12.03	0.05	0.044	88	0.046	92	0.045	90	0.0450	90.00	0.001	2.222
8.	Fenthion	12.25	0.05	0.045	90	0.044	88	0.043	86	0.0440	88.00	0.001	2.273
9.	Chlorpyrifos-methyl	12.44	0.05	0.048	96	0.046	92	0.045	90	0.0463	92.67	0.0015	3.237
10.	Parathion methyl	12.67	0.05	0.04	80	0.046	92	0.043	86	0.0430	86.00	0.003	6.977
11.	Fenitrothion	13.61	0.05	0.04	80	0.047	94	0.043	86	0.0433	86.67	0.0035	8.077
12.	Malathion	13.88	0.05	0.041	82	0.046	92	0.048	96	0.0450	90.00	0.0036	8.000
13.	Chlorpyrifos	14.25	0.05	0.048	96	0.045	90	0.047	94	0.0467	93.33	0.0015	3.214
14.	Parathion	14.43	0.05	0.043	86	0.048	96	0.046	92	0.0457	91.33	0.0025	5.474
15.	Chlorfenvinfos	15.95	0.05	0.045	90	0.046	92	0.041	82	0.0440	88.00	0.0026	5.909
16.	Quinolphos	16.42	0.05	0.043	86	0.046	92	0.046	92	0.0450	90.00	0.0017	3.778
17.	Fenamiphos	17.86	0.05	0.043	86	0.046	92	0.048	96	0.0457	91.33	0.0025	5.474
18.	Profenofos	18.58	0.05	0.046	92	0.042	84	0.044	88	0.0440	88.00	0.002	4.545
19.	Ethion	20.93	0.05	0.04	80	0.043	86	0.041	82	0.0413	82.67	0.0015	3.629
20.	Trizophos	21.6	0.05	0.043	86	0.04	80	0.046	92	0.0430	86.00	0.003	6.977
21.	Edfinphos	21.99	0.05	0.04	80	0.046	92	0.047	94	0.0443	88.67	0.0038	8.571
22.	Anilophos	24.15	0.05	0.04	80	0.044	88	0.043	86	0.0423	84.67	0.0021	4.961
23.	Phosalone	25.74	0.05	0.046	92	0.043	86	0.047	94	0.0453	90.67	0.0021	4.632

4.2.5 Selectivity/ Sensitivity

NIST library of Gas chromatography mass spectrometry was used to confirm the presence of pesticides taken for study. Spectra matched with reference spectra of NIST. Presence of pesticides was confirmed by their fragmentation pattern matches with reference spectra of NIST. Some of the reference spectra of pesticide of the group like chlorpyrifos and matrix blank chilli are given in figure 3 and figure 4 respectively. Table 3 shows mass fragmentation pattern of each pesticide for confirmation in GC-MS. Matrix blank sample of chilli was inspected in GC-FPD for the presence of interfering peaks in close to target retention times observed for all pesticides taken for study.

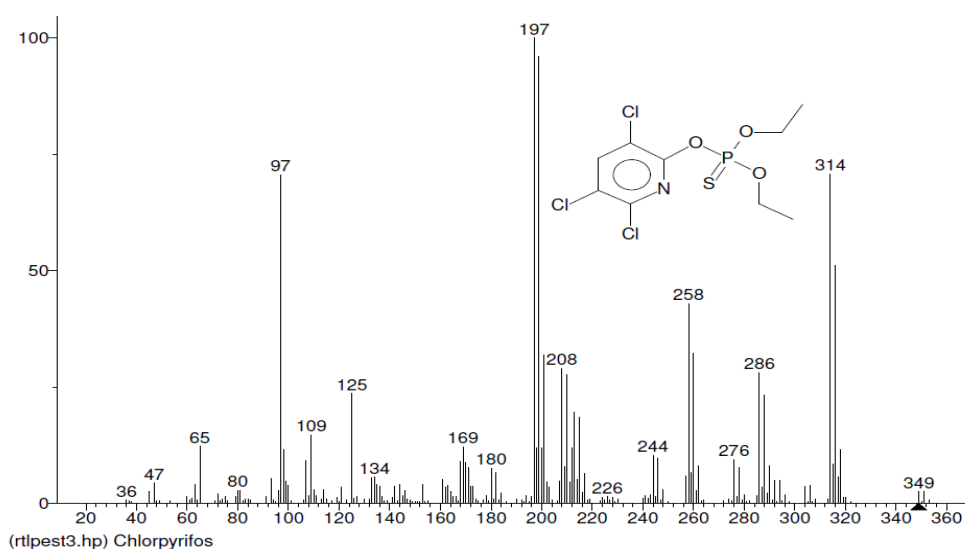


Fig.3 Shows, structure and mass fragmentation pattern of Chlorpyrifos

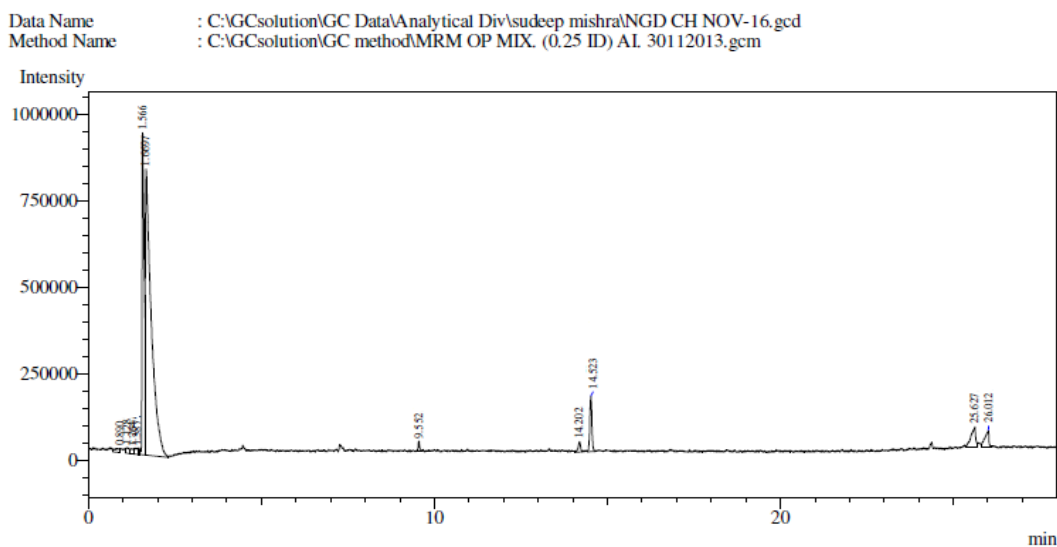


Fig. 4. GC- FPD chromatogram representing matrix of chilli sample

Table-3. Fragmentation pattern of 23 organophosphorus pesticides taken for study.

S.No	Organophosphorous Pesticides	Qualifier Ions (m/z)			S.No	Organophosphorous Pesticides	Qualifier Ions (m/z)		
1.	Anilophos	226	125	-	2.	Trizophos	257	161	-
3.	Chlorfenvinfos	323	269	267	4.	Fenitrothion	277	260	109
5.	Chlorpyrifos	314	197	97	6.	Phosalone	367	182	-
7.	Chlorpyrifos-methyl	286	125	-	8.	Paraxon-methyl	125	109	263
9.	Dichlorvos	220	109	185	10.	Fenamiphos	303	154	80
11.	Ethion	231	153	-	12.	Edfinphos	310	173	-
13.	Malathion	173	158	-	14.	Dimetoate	125	87	-
15.	Parathion methyl	263	233	125	16.	Diazinone	304	179	152
17.	Monocrotophos	192	164	127	18.	Fenthion	278	125	93
19.	Phorate	260	231	75	20.	Parathion	194	109	67
21.	Profenofos	337	139	97	22.	Phosphomidon	264	127	72
23.	Quinolphos	157	146	118					

Repeatability and Reproducibility

The method was analysed for recovery studies, which was repeated for three times are R1, R2 and R3. Their Mean (M), Standard Deviation (SD) and Relative Standard Deviation (RSD) of each pesticide were calculated as shown in Table 2. Similarly from table 4. three repeatable injections for 23 organophosphorus pesticide at 0.2 ppm, shows that area of three injections of pesticides mixture are repeatable with relative standard deviation lies from 0.4 for parathion methyl and chlorpyrifos, to 4.2 for paraxon methyl and anilophos. It was observed that all the three injections are repeatable. with Relative Standard Deviations lies \leq 5. Instrument injection precision was tested for both retention time and peak area for all target compounds by subsequent injections (n=3) of low concentration level (0.01) standard solution mixture for GC-FPD. Instrument injection precision for retention time was below 0.5% for all compounds. According to SANCO requirements, $<20\%$ was set as acceptance criteria for the target compounds and matrices.

Table-4. Shows Repeatability, Standard Deviation (SD), Relative Standard Deviation (RSD) of Organophosphorus pesticides at 0.2 ppm concentration.

Pesticide OP Mixture	RT	Area1	Area2	Area3	Avg area		
		Conc- 0.2 ppm				SD	RSD
Dichlorvos	5.40	2506197.9	2608334.2	2555469.7	2556667.3	51078.7	2.0
Monochrotofos	8.96	487739.5	505834.0	498669.0	497414.2	9112.3	1.8
Phorate	9.34	4724919.0	4773103.5	4540782.5	4679601.7	122611.2	2.6
Dimethoate	10.00	3292061.5	3294894.1	3188802.0	3258585.9	60451.2	1.9
Diazinon	10.50	5317957.4	5308985.2	5265212.0	5297384.9	28221.4	0.5
Paraxon Methyl	11.24	248347.5	263941.8	269776.3	260688.5	11078.6	4.2

Phosphomidon	12.03	349080.5	366515.8	376328.1	363974.8	13800.4	3.8
Chlorpyrifos Methyl	12.35	3404752.2	3385426.1	3425397.1	3405191.8	19989.1	0.6
Parathion Methyl	12.69	4499740.4	4534604.4	4503279.0	4512541.3	19189.0	0.4
Fenitrothion	13.62	4487551.2	4424437.3	4392251.9	4434746.8	48478.9	1.1
Malathion	13.85	3573118.4	3488960.5	3485294.9	3515791.3	49680.6	1.4
Chlorpyrifos	14.14	4249335.4	4222436.7	4216471.6	4229414.6	17507.9	0.4
Fenthion	14.40	5538473.6	5506928.0	5460041.3	5501814.3	39465.4	0.7
Parathion	14.57	5393547.9	5331008.6	5292156.3	5338904.3	51154.9	1.0
Chlorfenvinfos	16.06	1206036.9	1126814.2	1120510.7	1151120.6	47663.2	4.1
Quinalphos	16.37	5694740.5	5471337.9	5546732.8	5570937.1	113651.1	2.0
Fenamifos	18.06	1941495.9	1798367.5	1847255.5	1862373.0	72751.9	3.9
Profenofos	18.52	941377.0	932606.8	949488.8	941157.5	8443.1	0.9
Ethion	20.87	8709276.9	8523055.0	8518031.1	8583454.3	108994.5	1.3
Triazofos	21.65	4629673.7	4434306.7	4451260.1	4505080.2	108233.6	2.4
Edifinfos	22.09	341064.2	319302	321224.3	327196.83	12047.8921	3.7
Anilofos	24.21	1847951.1	1737264.9	1707005.7	1764073.9	74198.7	4.2
Phosalane	24.80	3495204.4	3361897.5	3325005.2	3394035.7	89535.4	2.6

CONCLUSION

Study focuses on (i) to develop validated method for the qualitative and quantitative estimation of Organophosphorus pesticides residues in chilli, using Gas Chromatography techniques (GC- FPD/MS) to reach lower limit of detection (below maximum residue limit). (ii) screening of different pesticides commonly used in the agricultural sector (iii) to monitor the persistence of pesticide residue in chilli. It would be better that we would develop new qualitative and quantitative method to detect common pesticide residue by GC which would be more sensitive and reliable. From this study, we will ensure that pesticides risk for human consumption are above or below MRL's set by European Commission. Robust analytical methods was validated for carrying out both research and monitoring programmes and thus for defining limitations and supporting enforcement of regulations. Reproducible analytical methods are required to allow the effective separation, selective identification and accurate quantification of pesticide analytes at low levels in food-stuffs including foods of plant and animal origin. Advances in sample preparation will minimise laboratory solvent use and hazardous waste productions, save manpower and time. It will reduce the cost in processing of samples. Method would be utilized by laboratory to detect pesticide residue level both qualitatively and quantitatively from vegetables and help in the management of pesticide poisoning. This approach will save time of food testing laboratories for the confirmation of pesticide both qualitative and quantitative.

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