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Uncertanity Determination of 23 Organophosphorus Pesticides Residues in Chilli fruit by Gas Chromatography, Flame Photometric Detector (FPD).

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ABSTRACT

The aim of this study is to estimate uncertainties associated with measurement of 23 organophosphorus pesticide residues in chilli. Exploration and evaluation of all these uncertanty sources are complicated and impractical. Study conducted to evaluate uncertainties of three basic analytical steps (First relative standard uncertainty due to purity of analytical standards, Uncertainty due to weighing of analytical CRM, Uncertainty associated with precision i.e repeatability. Uncertainty is important step for method development process. Combined uncertainty was determined at 0.05 mg/kg level for all the pesticides taken under study as per the statistical procedure of the EURACHEM/CITAC Guide CG 4.

To calculate the total uncertainty, relative uncertainty due to purity of standard, weighing and precision are considered. Total uncertainty is the sum of the square of relative uncertainty due to purity of standard, weighing, precision and their squareroot. Expanded uncertainty is twice of combined uncertainty at 95% confidence level. Combined uncertainty values lies between 0.00141-0.00499. *The expanded uncertainty of the pesticides falls under three ranges viz., (a) ≤10% (b) 11–15% and (c) 15–20% . Percent uncertainty of all the pesticides taken for study falls below ≤10%. Percent uncertainty value lies between 3-10 percent. Percent uncertainty value for edifenfos is highest and lowest for monocrotofos, phosphomidon and fenthion*

Keywords: CRM, Chilli, GC-FPD, QuEChER, Uncertanity.

I. INTRODUCTION

Uncertanity is one of the most important parameters of method validation which is defined as “a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand” [1-2]. By various analytical steps during the experiment, uncertainty originates from many sources such as sampling, matrix effect, uncertainty due to masses and volumeric equipment, reference standards, approximations, assumptions are incorporated in the method and its procedure and variations. [1&3]. Uncertainty of each analytical step consists of its random and systematic component (“error”) which are quantified and incorporated into the combined standard uncertainty. In any analytical method each uncertainty is calculated independently to obtain its contribution to over all uncertainty. To establish combined uncertainty of any method, the contribution of all other uncertainty sources are considered. From combined uncertainty, expanded uncertainty is calculated and confidence level within which an analytical results lie [1&4]. There are many potential sources of uncertainty which arise from individual phases of described multi residue method. Besides all gravimetric and volumetric steps (sample weighing, dilution of sample extracts, uncertainty of volume of GPC loop, etc.) there are many other operations and factors (evaporation of sample extracts, temperature, etc.) which contribute to the overall uncertainty. As per the international protocols, analytical methods are developed to controls regulation limits for food and feeds. Analytical methods performance characteristics such as, ruggedness, sensitivity, linearity, limit of detection etc [5-6]. Analytical testing laboratory shall apply procedure for uncertainty estimation. Analytical results cannot be only as a separate value but uncertainty information shall also be given in test reports as per EURACHEM/CITAC document [7], for estimation of combined standard uncertainty, bottom up approach can be used. Calculation of uncertainty for each step of the procedure contribute total uncertainty in the results. From such study, more significant steps and steps to be neglected are easily known. As per the published ISO 21748:2004 [8], more practically ‘‘top down’’ approach is recommended which shows that uncertainty measurement using data from inter lab studies performs as per ISO 5725-2[repeatability, reproducibility, standard deviation and measurement uncertainty] [9].Many papers are published during recent decade which are concerned to uncertainty measurement of organic contaminants [10-14]. Diaz et al [14] described the bottom up approach for calculation of uncertainty of nonyl phenol in water by GC-MS detection. Bottom up approach was also applied for the calculation of uncertainty from organophosphorous and organochlorine pesticides present in cucumber [3].

III. MATERIALS AND METHOD

2.1.Chemicals Reagents and Samples

All solvents used were HPLC grade. acetone, acetonitrile, ethyl acetate, methanol, and n-hexane were purchased from Merck Germany. Florisil, anhydrous sodium sulphate, sodium chloride, glass wool, celite 545, charcoal, magnesium oxide, cotton, filter paper, and magnesium sulphate anhydrous were purchased from Merck Germany. Primary Secondary Amine i.e. PSA (40 μm, Bondesil) sorbent was purchased from Agilent Technologies. C-18 silica sorbent used in this study was of Supelco and procured from Sigma Aldrich. Chilli fruit free of pesticides were obtained from organic farms of Satna district of Madhya Pradesh, India.

2.2 Certified Reference Material (CRM)and Standard Stock Solutions

All pesticide standards were of high purity above 98%. Certified reference materials (CRM) were procured from Accustandard Inc. (USA) and Sigma Aldrich for all the pesticides under study i.e. 23-organophosphorous pesticides (Anilophos, Chlorfenvinfos, Chlorpyrifos, Chlorpyrifos-methyl, Dichlorvos, Ethion, Malathion, Parathion methyl, Monocrotophos, Phorate, Profenofos, Quinolphos, Trizophos, Fenitrothion , Phosalone, Paraxon-methyl, Fenamiphos, Edfinphos, Dimetoate, Diazinon, Fenthion, Parathion and Phosphomidon). These pesticides are widely used by local farmers in vegetable cultivation. The use of high purity reagents and solvents help to minimize interference problems. CRM of individual pesticide was weighed directly in clean and dried standard volumetric flask of 10 ml. on analytical balance pan (Mettler, Toledo) maximum up to 4mg, dissolved in few drops of HPLC grade acetone which was further made up to the mark of standard volumetric flask with HPLC grade hexane. Standard solutions of CRM were prepared at seven different concentration levels of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/kg all gave good resposnse for FPD detector which were considered for study. All these working standard solutions of a mixture of pesticides were prepared for calibration and recovery tests.

III.ANALYTICAL METHODS

3.1 Sample Extraction and Clean up

Samples were prepared according to the QuEChERS (quick, easy, cheap, effective, rugged and safe) method [15] with some modifications. Sample preparation has the following steps: chilli fruit was finely chopped and homogenized in a mixer grinder (Bajaj make). Fifteen gram of thoroughly homogenized sample weighed into a 50 ml fluoroethylenepropylene (FEP) centrifugation tube (Tarson make), and 30 ml of Ethyl acetate was added and shaken for 1 min Vortex shaker. Ten gram anhydrous Na_2SO_4 was added and shaken vigorously for 1 min by hand. The tubes were centrifuged at 3,450 relative centrifugal force at 5,000 rpm on the centrifuge mechine (Remi, India) at about 5 minutes. Cleanup was performed according to Lehotay (2007) [16]. 6 ml extract was transferred from the upper layer into a 15 ml FEP tube, and 0.9 g anhydrous $MgSO_4$, 0.25 g PSA and 0.25 g Activated charcoal to remove pigments were added and shaken vigorously for 1 min by vortex shaker. The tubes were centrifuged at 5,000 rpm (Remi, India) for 5 min. The supernatant 4 ml was dried and finally made up to 1 ml for injection in GC-FPD.

3.2 Gas Chromatography – Flame Photometric Detector (GC-FPD) and data processing

Chromatographic separations were carried out 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 pesticides. 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. All chromatographic data were processed using GC solution software of Shimadzu make GC-FPD (GC-QP 2010 model) with AOC-20S Auto Sampler.

IV. DETERMINATION OF UNCERTANITIES DURING VALIDATION OF QUANTITATIVE CHROMATOGRAPHY METHOD

The measurement uncertainty was calculated as per EURACHEM/CITAC and quantifying uncertainty for pesticide residue analysis in chilli. Uncertanities arise during the experiment are as follows-

1. Standard solution preparation

1.1 Purity of standards

1.2 Weight of standards

1.3 Volumetric flask volume measurement.

1.4 Volume measurement using micropipette

2. Calibration curve preparation

3. Sample Preparation.

3.1 Weighing balance

3.2 Volume

4. Repeatability

5. Bias (Recovery)

6. Uncertainty in CRM purity

7. Uncertainty in preparation of std. solution

8. Uncertainty in GC response

V. RESULTS AND DISCUSSIONS

Uncertainty arise during method validation and analysis of pesticide residues in chilli. The aim of this study was to estimate uncertainties associated with measurement of pesticide residues in chilli involved three basic steps:

(i) identification of of uncertainty potential sources.

(ii) quantification of uncertainty components.

(iii) calculation of the combined standard uncertainty.

Exploration and evaluation of all these uncertanty sources are complicated and impractical. Therefore the decision was made to evaluate uncertainties of three basic analytical steps (First relative standard uncertainty (U1) due to purity of analytical standards, Uncertainty due to weighing (U2) of analytical CRM, Uncertainty associated with precision (U3) i.e repeatability. Uncertainty is important step for method development process. Combined uncertainty (U) was determined at 0.05 mg/kg level for all the pesticides taken under study as per the statistical procedure of the EURACHEM/CITAC Guide CG 4.[7]

5.1 Identification of Uncertainty Sources

5.1.1Repeatability

5.1.2 Recovery

5.1.3 Uncertainty in CRM purity

5.1.4 Uncertainty in weighing

5.1.5 Uncertainty in preparation of std. solution

5.1.6 Uncertainty in GC response

5.1.7 Uncertainty in sample homogeneity

5.2 Measurand

$$\text{ppm conc.} = \frac{\text{area of sample}}{\text{area of standard}} \text{ X conc. of standard X dilution X } \frac{1}{\text{sample weight}}$$

5.3 Quantification of Uncertainty Sources

a. Uncertainty of volumetric flask (10ml). Calibrated, class A glasswares were used, so uncertainty due to glasswares can be neglected.

b. Uncertainty of micro pipette; calibrated pipettes of 1000 and 200 micro litre were used, so uncertainty due to micro pipette can be neglected.

c. Uncertainty in GC response; Uncertainty in linearity of response is in given concentration range has been included in the precision study. Hence no separate allowance is necessary.

d. Uncertainty in sample homogeneity; it can be assumed that pesticide residues are uniformly distributed in the sample. Hence the uncertainty due to sample homogeneity can be ignored.

5.4 Main cause of uncertainty

5.4.1 First relative standard uncertainty (U1) due to purity of analytical standards.

5.4.2 Uncertainty due to weighing (U2) of analytical CRM.

5.4.3 Uncertainty associated with precision (U3) i.e repeatability.

5.4.1 Uncertainty by purity of analytical standards (U1)

From all 23 pesticides with their specific purity percent have uncertainty mentioned in the certificate of purity. Rectangular distribution were considered as purity certificate which indicates lack of any confidence level. So by formula, first standard uncertainty SU1 is-.

SU1 = (u (x) /√3) where u(x) is the uncertainty value given in the certificate for purity of CRM, and due to rectangular distribution, uncertainty is divided by √3. From uncertainty table 1, uncertainty of all pesticides CRM purity are almost same i.e 0.5% which is converted to (0.005).

whereas relative standard uncertainty (U1) derived according to the equation:.

U1 = (SU1 × 100)/% purity

From table 1., the values of relative standard uncertainty were found close to standard uncertainty. Highest U1 value was found for trizofos, fenamifos and monocrotofos whereas rest values were almost same. The values of U1 lies between 0.0029-0.0030.

Table-1. Uncertanity Calculations of Purity of Standards (Certified Reference Material).

S.No.	Pesticide	Purity % of CRM	Uncertainity in certificate	Standard Uncertanity (SU1)	Relative Standard Uncertainty (U1)
1.	Dichlorvos	98.5	0.005	0.0029	0.0029
2.	Monocrotophos	96	0.005	0.0029	0.003
3.	Phorate	99.4	0.005	0.0029	0.0029
4.	Dimetoate	96	0.005	0.0029	0.003
5.	Diazinon	98.9	0.005	0.0029	0.0029
6.	Paraxon-methyl	97.2	0.005	0.0029	0.003
7.	Phosphomidon	98.9	0.005	0.0029	0.0029
8.	Fenthion	99	0.005	0.0029	0.0029
9.	Chlorpyrifos-methyl	99.7	0.005	0.0029	0.0029
10.	Parathion methyl	99.7	0.005	0.0029	0.0029
11.	Fenitrothion	99.5	0.005	0.0029	0.0029
12.	Malathion	99.5	0.005	0.0029	0.0029

13.	Chlorpyrifos	99.3	0.005	0.0029	0.0029
14.	Parathion	98.8	0.005	0.0029	0.0029
15.	Chlorfenvinfos	99.5	0.005	0.0029	0.0029
16.	Quinolphos	99.3	0.005	0.0029	0.0029
17.	Fenamiphos	96	0.005	0.0029	0.003
18.	Profenofos	99.2	0.005	0.0029	0.0029
19.	Ethion	98	0.005	0.0029	0.0029
20.	Trizophos	97.8	0.005	0.0029	0.003
21.	Edfinphos	98.5	0.005	0.0029	0.0029
22.	Anilophos	98.4	0.005	0.0029	0.0029
23.	Phosalone	98.6	0.005	0.0029	0.0029

5.4.2Uncertainty of weighing (U2)

From table-2, uncertainty weighing shows that weight of standard taken between 1-2 gm with 0.001 uncertanity of weighing balance. To estimate relative standard uncertainty due to weighing (U2) normal distribution was considered by equation:

U2 = (0.0001/2)/Wi where Wi is the weight of the pesticide standard weighed using precision analytical balance, 0.0001 is the value of uncertainty at 95% confidence level taken from the valid calibration certificate of balance. Considering normal distribution, the uncertainty of the balance was divided by taking two. The relative standard uncertainty values lies between 0.002916-0.0046189. From the table 2, the values of U2 for chlorfenvinfos and paraxon methyl were lowest whereas highest for dichlorvos.

Table-2. Uncertanity calculations of weighing.

S.No.	Pesticide	Weighted standard	Uncertainty of balance	Standard Uncertanity (SU2)	Relative Standard Uncertanity (U2)
1.	Dichlorvos	1.25	0.0001	5.77367E-05	0.0046189
2.	Monocrotophos	1.36	0.0001	5.77367E-05	0.0042453
3.	Phorate	1.54	0.0001	5.77367E-05	0.0037491
4.	Dimetoate	1.67	0.0001	5.77367E-05	0.0034573
5.	Diazinon	1.32	0.0001	5.77367E-05	0.004374
6.	Paraxon-methyl	1.98	0.0001	5.77367E-05	0.002916
7.	Phosphomidon	1.56	0.0001	5.77367E-05	0.0037011
8.	Fenthion	1.87	0.0001	5.77367E-05	0.0030875
9.	Chlorpyrifos-methyl	1.78	0.0001	5.77367E-05	0.0032436
10.	Parathion methyl	1.46	0.0001	5.77367E-05	0.0039546

11.	Fenitrothion	1.56	0.0001	5.77367E-05	0.0037011
12.	Malathion	1.35	0.0001	5.77367E-05	0.0042768
13.	Chlorpyrifos	1.67	0.0001	5.77367E-05	0.0034573
14.	Parathion	1.4	0.0001	5.77367E-05	0.0041241
15.	Chlorfenvinfos	1.98	0.0001	5.77367E-05	0.002916
16.	Quinolphos	1.68	0.0001	5.77367E-05	0.0034367
17.	Fenamiphos	1.59	0.0001	5.77367E-05	0.0036312
18.	Profenofos	1.67	0.0001	5.77367E-05	0.0034573
19.	Ethion	1.37	0.0001	5.77367E-05	0.0042144
20.	Trizophos	1.49	0.0001	5.77367E-05	0.0038749
21.	Edfinphos	1.67	0.0001	5.77367E-05	0.0034573
22.	Anilophos	1.32	0.0001	5.77367E-05	0.004374
23.	Phosalone	1.37	0.0001	5.77367E-05	0.0042144

5.4.3 Uncertainty associated with precision (U3)

Uncertainty associated with precision from table 3. shows that three replicate recovery for test mixture of 23 organophosphorus pesticides and their mean value , standard deviation, relative standard deviation were calculated. During sample processing steps, errors caused at extraction, clean up, and GC analyses steps were approximated by standard deviations (s), calculated from triplicate determinations of analytes expressed as repeatability by equation:

$U3 = s/(\sqrt{n} \times x)$........................ (4)

where standard deviation (s) is obtained from the recovery study, n is the number of replications and x is the mean value of the concentration recovered. Highest possibility was for phosphomidon i.e 0.013 whereas lowest repeatability for 0.0341 chlorfenvinfos.

To calculate the total uncertainty , Relative uncertainty due to purity of standard(U1), due to weighing(U2) and precision are considered. For calculating combined uncertainty, the sum of the square root of U1, U2 and U3 are taken. The combined uncertainty (U) was calculated by equation:

$U = x\,[(U1)^2+(U2)^2+(U3)^2]^{1/2}$

Expanded uncertainty (2U) was twice of combined uncertainty (U) at 95% confidence level. From table no.4, combined uncertainty values lies between 0.00141-0.00499. The factor is also included to find the uncertain value i.e mean recovery amount is multiplied by expanded uncertainty value. The factor value obtained is the uncertain value of recovered amount. Also percent uncertainty value is calculated by dividing factor value from recovered amount value and multiplied by 100. From the table 4. The expanded uncertainty of the pesticides was under three ranges viz., (a) ≤10% (b) 11–15% and (c) 15–20% . Percent uncertainty of all the pesticides taken for study was found below ≤10%. Percent uncertainty value lies between 3-10 percent. Percent uncertainty value for edifenfos was highest and lowest for monocrotofos, phosphomidon and fenthion

Table-3. Recovery, Standard Deviation (S.D), Relative Standard Deviation (RSD) and Uncertanity associated with precision of Organophosphorus pesticides from spiked chilli matrix.

S.NO.	Pesticide	RT	Spiking Conc. (ppm)	Amount Recovered (R1)	Amount Recovered (R2)	Amount Recovered (R3)	Mean Recovery Amount	S.D.	R.S.D	Standard Uncertainty Precision(SU3)	Relative Standard Uncertainity
1.	Dichlorvos	5.49	0.05	0.043	0.045	0.04	0.0427	0.0025	5.859	0.0014	0.03278689
2.	Monocrotophos	9.16	0.05	0.043	0.044	0.045	0.044	0.001	2.273	0.0006	0.01363636
3.	Phorate	9.44	0.05	0.045	0.042	0.043	0.0433	0.0015	3.462	0.0009	0.02078522
4.	Dimetoate	9.86	0.05	0.047	0.046	0.043	0.0453	0.0021	4.632	0.0012	0.02649007
5.	Diazinon	10.43	0.05	0.043	0.048	0.046	0.0457	0.0025	5.474	0.0014	0.03063457
6.	Paraxon-methyl	11.22	0.05	0.043	0.04	0.043	0.042	0.0017	4.048	0.0010	0.02380952
7.	Phosphomidon	12.03	0.05	0.044	0.046	0.045	0.045	0.001	2.222	0.0006	0.01333333
8.	Fenthion	12.25	0.05	0.045	0.044	0.043	0.044	0.001	2.273	0.0006	0.01363636
9.	Chlorpyrifos-methyl	12.44	0.05	0.048	0.046	0.045	0.0463	0.0015	3.237	0.0009	0.01943844
10.	Parathion methyl	12.67	0.05	0.04	0.046	0.043	0.043	0.003	6.977	0.0017	0.03953488
11.	Fenitrothion	13.61	0.05	0.04	0.047	0.043	0.0433	0.0035	8.077	0.0020	0.04618938
12.	Malathion	13.88	0.05	0.041	0.046	0.048	0.045	0.0036	8.000	0.0021	0.04666667
13.	Chlorpyrifos	14.25	0.05	0.048	0.045	0.047	0.0467	0.0015	3.214	0.0009	0.01927195
14.	Parathion	14.43	0.05	0.043	0.048	0.046	0.0457	0.0025	5.474	0.0014	0.03063457
15.	Chlorfenvinfos	15.95	0.05	0.045	0.046	0.041	0.044	0.0026	5.909	0.0015	0.03409091
16.	Quinolphos	16.42	0.05	0.043	0.046	0.046	0.045	0.0017	3.778	0.0010	0.02222222
17.	Fenamiphos	17.86	0.05	0.043	0.046	0.048	0.0457	0.0025	5.474	0.0014	0.03063457
18.	Profenofos	18.58	0.05	0.046	0.042	0.044	0.044	0.002	4.545	0.0012	0.02727273
19.	Ethion	20.93	0.05	0.04	0.043	0.041	0.0413	0.0015	3.629	0.0009	0.02179177

20.	Trizophos	21.6	0.05	0.043	0.04	0.046	0.043	0.003	6.977	0.0017	0.03953488
21.	Edfinphos	21.99	0.05	0.04	0.046	0.047	0.0443	0.0038	8.571	0.0022	0.0496614
22.	Anilophos	24.15	0.05	0.04	0.044	0.043	0.0423	0.0021	4.961	0.0012	0.02836879
23.	Phosalone	25.74	0.05	0.046	0.043	0.047	0.0453	0.0021	4.632	0.0012	0.02649007

Table-4. Total uncertainty combined uncertainty, expanded uncertainty and uncertainty value.

S. No.	Pesticide	Mean Rec. Amount	Relative Standard Uncertanity (U1) of Purity of Standard	Relative Standard Uncertanity (U2) of weighing	Relative Standard Uncertanity Precision(U3)	Combined Uncertanity	Expanded Uncertanity	Uncertanity value (Recovery amt x expanded uncertainty)	Percent Uncertanity
1.	Dichlorvos	0.0427	0.0029	0.0046	0.03278689	0.0332	0.066	0.003	7
2.	Monocrotophos	0.044	0.0030	0.0042	0.01363636	0.0146	0.029	0.001	3
3.	Phorate	0.0433	0.0029	0.0037	0.02078522	0.0213	0.043	0.002	4
4.	Dimetoate	0.0453	0.0030	0.0035	0.02649007	0.0269	0.054	0.002	5
5.	Diazinon	0.0457	0.0029	0.0044	0.03063457	0.0311	0.062	0.003	6
6.	Paraxon-methyl	0.042	0.0030	0.0029	0.02380952	0.0242	0.048	0.002	5
7.	Phosphomidon	0.045	0.0029	0.0037	0.01333333	0.0141	0.028	0.001	3
8.	Fenthion	0.044	0.0029	0.0031	0.01363636	0.0143	0.029	0.001	3
9.	Chlorpyrifos-methyl	0.0463	0.0029	0.0032	0.01943844	0.0199	0.040	0.002	4
10.	Parathion	0.04	0.0029	0.0040	0.039534	0.0398	0.080	0.003	8

	methyl	3			88				
11.	Fenitrothion	0.0433	0.0029	0.0037	0.04618938	0.0464	0.093	0.004	9
12.	Malathion	0.045	0.0029	0.0043	0.04666667	0.0470	0.094	0.004	9
13.	Chlorpyrifos	0.0467	0.0029	0.0035	0.01927195	0.0198	0.040	0.002	4
14.	Parathion	0.0457	0.0029	0.0041	0.03063457	0.0310	0.062	0.003	6
15.	Chlorfenvinfos	0.044	0.0029	0.0029	0.03409091	0.0343	0.069	0.003	7
16.	Quinolphos	0.045	0.0029	0.0034	0.02222222	0.0227	0.045	0.002	5
17.	Fenamiphos	0.0457	0.0030	0.0036	0.03063457	0.0310	0.062	0.003	6
18.	Profenofos	0.044	0.0029	0.0035	0.02727273	0.0276	0.055	0.002	6
19.	Ethion	0.0413	0.0029	0.0042	0.02179177	0.0224	0.045	0.002	4
20.	Trizophos	0.043	0.0030	0.0039	0.03953488	0.0398	0.080	0.003	8
21.	Edfinphos	0.0443	0.0029	0.0035	0.0496614	0.0499	0.100	0.004	10
22.	Anilophos	0.0423	0.0029	0.0044	0.02836879	0.0289	0.058	0.002	6
23.	Phosalone	0.0453	0.0029	0.0042	0.02649007	0.0270	0.054	0.002	5

VI. CONCLUSION

The method followed for all pesticides taken for study is efficient in determining pesticides from chilli matrix. The analytical method developed for separation of 23 organophosphorus pesticides in chilli matrix using QuEChER technique is for extraction and cleanup. Final analysis of the pesticide taken for study was done by GC-FPD and confirmation of pesticides by GC-MS. The method is validated properly following guidelines of AOAC. The results obtained by the method are specific, accurate and reproducible. Uncertanity arise by various steps of the method are rectified and calculated according to SANCO guidelines.

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