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Uncertanity of Measurement During Estimation of 23 Organophosphorus Pesticides Residue Present in Bottle Gourd

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Abstract

The study presents the assessment of uncertainty calculation generated, within the analysis of selected 23 organophosphrus pesticides residues of bottle guard. The samples were prepared by using a modified quick, easy, cheap, effective, rugged and safe (QuEChERS) analytical protocol. Multiresidue method used for analysis of samples consisted of (i) acetonitrile extraction, (ii) PSA/C18 clean-up and (iii) identification/quantification of residues by GC utilizing either (nitrogen–phosphorus) or mass-selective detectors (quadrupole analyzer) were evaluated. Major sources like weighing of standard, purity of certified reference material, precision study i.e repeatability and standard solution preparation for calculation of uncertainty of method, was considered. Identification of uncertainty sources, quantification of uncertainty sources and calculation of combined uncertainty are steps for calculation of uncertainty. All the individual uncertainty calculated are combined, later converted to expanded uncertainty.

Keywords: Bottle guard; Uncertanity; Combined Uncertanity; Expanded Uncertanity; Pesticide.

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Inroduction

Estimated uncertainty of measurement is an integral part of analytical results. This paper focuses on major sources of errors concern with pesticide residue analysis. The sampling, sample processing and analysis influence the uncertainty and accuracy of analytical data. Their combined effects should be considered in deciding on the reliability of the results. (Stanisław Walorczyk, March 2014). The estimation of the uncertainty for any analytical methods is necessary for establishing the comparability of results. Multiresidue analytical methods lack very often of information about uncertainty of results, when results are compared with maximum residue levels (MRL) established by regulations. Identification and estimation of each uncertainty source allows laboratories to establish the accuracy of results and to balance with time-consuming and costs (L.Cuadros-Rodrıíguez, et al., 2002).

Uncertainty associated with analytical results make available as per ISO/IEC 17025 accrediated laboratories (ISO/IEC 2005). To calculate uncertainty, firstly measurand is specified, possible uncertainty sources are identified and quantified, finally combined uncertainty is calculated (EURACHEM/CITAC, 2000). Estimating the uncertainty for analytical measurements, basically two methods bottom-up and top-down methods

are applied. In the bottom-up method, analytical procedures are divided into individual components or steps. Their standard uncertainties are estimated and summed up together to form the combined uncertainty. Codex Committees on Methods of Analysis and Sampling (CCMAS) are working on the development of guidelines for estimation and interpretation of uncertainty of measurement results (CAC, 2010).

Materials and Methods

Total 23 Organophosphorus pesticides i.e 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 were taken for study. All pesticides taken were good sensitive for GC-FPD detector. In todays contrast pesticides are frequently used by farmers to protect their crops from insects, pests and weeds. Among various crops, vegetables are highly sprayed by pesticides. All pesticides standard of higher purity were procured from Sigma Aldrich (Germany). For extraction of vegetables from bottle gourd acetonitrile solvent was used. For moisture removal from sample, sodium sulphate anhydrous and magnesium sulphate anhydrous were used. For cleaning the sample the samples Primary Secondary Amine (40 µm, Bondesil) and C-18 silica sorbent were used. All the chemicals and solvents used during analysis were purchased from Sigma Aldrich and Merck Germany. The use of high purity reagents and solvents is to minimise matrix interference and increase sensitivity and life of instrument. The bottle guard fruit should be free from any pesticides befor going for extraction and analysis.

GC-FPD (GC-QP 2010 model) Shimadzu make with AOC-20S Auto Sampler was used for analysis. DB-5MS fused silica capillary column (Agilent J&W GC column, 5% Phenylated methyl siloxane), was used for separation of 23 pesticides which is of 30 m length × 0.25 mm i.d. × 0.25 µm film thickness was used for screening and quantification of pesticide residues. The oven programming was set at 100°C for 2 min with a ramp of 25°C/min up to 200°C for 5 min., then 4°C/min ramp upto 230°C for 2 min and 20°C/min to final temperature of 280°C with a hold time of 5 min. The injector port temperature was 250°C and detector temperature was, 290°C. Injection volume 1.0 micro litre and 0.5 min equilibrium time. The instrument works in split mode of (10:1). Helium gas was used as makeup gas and carrier gas at a flow rate of 1.23 mL/min. H_2 and air for combustion of flame with flow of 85 ml/min and 110.0 ml/min respectively.

Certified Reference Materials (CRM) of pesticide of above 95% was weighed in clean volumetric flask of of 10 ml. The standard solution of about 100 ppm was prepared using HPLC grade acetone and hexane solvent. The working standard was prepared by serial dilution from standard stock solution which should be kept at -20°C. The calibration standard solution was prepared at seven different concentration levels of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/kg are considered for study. All working standard solutions of a mixture of pesticides were prepared for calibration and recovery tests.

Sample Preparation

The present environmental load of the pesticide residues is increasing day by day. It is important to determine the amount of pesticide residues in vegetable samples in and around Satna, Madhya Pradesh, India. Samples were prepared according to the QuEChERS (quick, easy, cheap, effective, rugged and safe) method [Anastassiades, M., *et al*., (2003)] with some modifications. The estimation of commonly used pesticides for a period of one year. Spinach was chopped and homogenized, 15 gm of homogenized sample was taken in 50 ml centrifugation tube and added 30 ml of Ethyl acetate later shaken for 1 min then 10 gram anhydrous Na_2SO_4 was added and shaken for 1 min. 6 ml extract used for ceaning up according to Lehotay (2007). 0.9 g anhydrous $MgSO_4$, 0.25 g PSA and 0.25 g Activated charcoal was used for cleaning to remove matrix effect of highly pigmented foods. The supernatant 4 ml of cleanup extract was dried and 1 ml n hexane was added for injection in GCFPD and GC-MS.

Theory of uncertainty estimation

Uncertanity is very important steps of test results performed in any accredited laboratory. Following EURACHEM/CITAC guidelines, calculation of measurement uncertainty of 23 organophosphorus pesticides in bottle gourd occurs during standard solution preparation purity of standards, weight sample and certified reference standard , repeatability of results, recovery percentage, purity of CRM and Gas Chromatography responses.

Uncertainty involves measurand specificity, identity of uncertainty sources, quantification of uncertainty sources. The standard deviation value of sources which can be directly used, declared uncertain value in certificate and confidence level can be used for calculating uncertainty. Standard uncertainty (u(x)), is standard deviation of values, combined standard uncertainty is the sum of the square and their square root of all uncertainties whereas expanded uncertainty is (U(x)) is calculated from a combined standard uncertainty and a coverage factor k. Relative standard uncertainty (u rel) value is obtained as the division of standard uncertainty u(x) and the value of x. Formulas of the uncertainty mentioned are given below.

Standard Uncertanity u(x)= standard deviation values

urel(x) = u(x)/x

Combined Uncertanity (u) = square root of ((x_* x) + (y_* y)+…)

Expanded Uncertanity U(x)= (u)* coverage factor

Results and Discussion

For estimation of 23 organophosphorus pesticide in bottle guard. The study was conducted and method of analysis was validated. During validation step there are many sources of uncertainty arises. Steps involved are identification of uncertainty sources, quantification of uncertainty sources and overall calculation of the combined standard uncertainty. Uncertanity consists of random and systematic errors. All the errors are quantified and included in the combined standard uncertainty. As per the statistical procedure of the EURACHEM/CITAC Guide CG-4 [Eurachem/CITAC Guide, 2000]. Many sources of uncertainty arise in multiresidue method due to gravimetric and volumetric steps. Major sources of uncertainty due to purity of CRM, weighing, recovery and repeatability of results. Combined uncertainty (U) was calculated at 0.05 mg/kg level.

Pesticides taken for study have their specific purity percent. Standard uncertainty by purity of analytical standards (U1) was calculated from uncertainty value given in the certificate. Uncertainty value u(x) is divided by √3 (rectangular distribution) so the formula is-. U1 = (u (x) /√3). From uncertainty table 1, uncertainty of all pesticides CRM purity are almost same i.e 0.05 which is converted to (0.05/√3).

The uncertainty of the weighing (U2) is taken during weighing of 1–2 mg of Certified Reference

Table 1: Shows the uncertainty calculation due to purity of certified reference standards.

S. No.	Pesticide Standard	Purity of Standard	Wt. std	Uncertainity	Uncertanity of Standard SU1	Relative Standard Uncertanity (U1)
1	Dichlorvos	98.5	1.25	0.25	0.1443	0.1465
2	Monocrotophos	96	1.36	0.25	0.1443	0.1504
3	Phorate	99.4	1.54	0.25	0.1443	0.1452
4	Dimetoate	96	1.67	0.25	0.1443	0.1504
5	Diazinon	98.9	1.32	0.25	0.1443	0.1459
6	Paraxon-methyl	97.2	1.98	0.25	0.1443	0.1485
7	Phosphomidon	98.9	1.56	0.25	0.1443	0.1459
8	Fenthion	99	1.87	0.25	0.1443	0.1458
9	Chlorpyrifos-methyl	99.7	1.78	0.25	0.1443	0.1448
10	Parathion methyl	99.7	1.46	0.25	0.1443	0.1448
11	Fenitrothion	99.5	1.56	0.25	0.1443	0.1451
12	Malathion	99.5	1.35	0.25	0.1443	0.1451
13	Chlorpyrifos	99.3	1.67	0.25	0.1443	0.1454
14	Parathion	98.8	1.4	0.25	0.1443	0.1461
15	Chlorfenvinfos	99.5	1.98	0.25	0.1443	0.1451
16	Quinolphos	99.3	1.68	0.25	0.1443	0.1454
17	Fenamiphos	96	1.59	0.25	0.1443	0.1504
18	Profenofos	99.2	1.67	0.25	0.1443	0.1455
19	Ethion	98	1.37	0.25	0.1443	0.1473
20	Trizophos	97.8	1.49	0.25	0.1443	0.1476
21	Edfinphos	98.5	1.67	0.25	0.1443	0.1465
22	Anilophos	98.4	1.32	0.25	0.1443	0.1467
23	Phosalone	98.6	1.37	0.25	0.1443	0.1464

Material. Considering normal distribution of weight is taken in weighing balance, the uncertainty value i.e 0.05 gm. Standard uncertainty is the uncertainty of weighing balance divided by normal distribution (2) and relative standard uncertainty is standard uncertainty divided by the weight of pesticide standard weighted using precision analytical balance of 0.05 uncertainty value at 95% confidence level. The calculation of uncertainty value occurs due to weighing of CRM are calculated in Table 2.

Uncertainty arises due to precision (U3) of 23 organophosphorus pesticides. Standard deviation and relative standard deviation of repeatability were calculated. Repeatability was calculated by equation: $U3 = s/(\sqrt{n} \times x)$ 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 (Table 3).

Table 2: Shows the uncertainty calculation due to weighing of certified reference standards.

S. No.	Pesticide Standard	Weight of Standard	Uncertainty in Weighing	Standard Uncertainty	Relative Standard Uncertainty (U2)
1	Dichlorvos	1.25	0.05	0.025	0.020
2	Monocrotophos	1.36	0.05	0.025	0.018
3	Phorate	1.54	0.05	0.025	0.016
4	Dimetoate	1.67	0.05	0.025	0.015
5	Diazinon	1.32	0.05	0.025	0.019
6	Paraxon-methyl	1.98	0.05	0.025	0.013
7	Phosphomidon	1.56	0.05	0.025	0.016
8	Fenthion	1.87	0.05	0.025	0.013
9	Chlorpyrifos-methyl	1.78	0.05	0.025	0.014
10	Parathion methyl	1.46	0.05	0.025	0.017
11	Fenitrothion	1.56	0.05	0.025	0.016
12	Malathion	1.35	0.05	0.025	0.019
13	Chlorpyrifos	1.67	0.05	0.025	0.015
14	Parathion	1.4	0.05	0.025	0.018
15	Chlorfenvinfos	1.98	0.05	0.025	0.013
16	Quinolphos	1.68	0.05	0.025	0.015
17	Fenamiphos	1.59	0.05	0.025	0.016
18	Profenofos	1.67	0.05	0.025	0.015
19	Ethion	1.37	0.05	0.025	0.018
20	Trizophos	1.49	0.05	0.025	0.017
21	Edfinphos	1.67	0.05	0.025	0.015
22	Anilophos	1.32	0.05	0.025	0.019
23	Phosalone	1.37	0.05	0.025	0.018

Table 3: Shows Recovery, Mean Recovery, Standard Deviation (S.D), and Relative Standard Deviation (RSD) of Organophosphorus pesticides from spiked bottle gourd matrix at 0.05 ppm.

S. No.	Pesticide	Spike Conc	Amount Recovered	Amount Recovered	Amount Recovered	Mean Recovery	S.D.	R.S.D
1	Dichlorvos	0.05	0.046	0.04	0.045	0.0437	0.0032	7.3626
2	Monocrotophos	0.05	0.044	0.048	0.042	0.0447	0.0031	6.8396
3	Phorate	0.05	0.04	0.038	0.04	0.0393	0.0012	2.9364
4	Dimetoate	0.05	0.043	0.045	0.042	0.0433	0.0015	3.5262
5	Diazinon	0.05	0.042	0.04	0.046	0.0427	0.0031	7.1602
6	Paraxon-methyl	0.05	0.043	0.043	0.042	0.0427	0.0006	1.3523
7	Phosphomidon	0.05	0.042	0.046	0.042	0.0433	0.0023	5.3285
8	Fenthion	0.05	0.043	0.041	0.046	0.0433	0.0025	5.8085
9	Chlorpyrifos-methyl	0.05	0.042	0.043	0.044	0.0430	0.0010	2.3256
10	Parathion-methyl	0.05	0.046	0.044	0.047	0.0457	0.0015	3.3460
11	Fenitrothion	0.05	0.044	0.043	0.04	0.0423	0.0021	4.9181

Total uncertainty is calculated by considering, relative uncertainty due to purity of standard (U1), due to weighing (U2) and precision (U3). 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 (Table 4).

S. No.	Pesticide	Spike Conc	Amount Recovered	Amount Recovered	Amount Recovered	Mean Recovery	S.D.	R.S.D
12	Malathion	0.05	0.039	0.042	0.04	0.0403	0.0015	3.7884
13	Chlorpyrifos	0.05	0.043	0.046	0.039	0.0427	0.0035	8.2313
14	Parathion	0.05	0.046	0.043	0.04	0.0430	0.0030	6.9767
15	Chlorfenvin-fos	0.05	0.043	0.044	0.046	0.0443	0.0015	3.4466
16	Quinolphos	0.05	0.045	0.042	0.041	0.0427	0.0021	4.8797
17	Fenamiphos	0.05	0.045	0.043	0.046	0.0447	0.0015	3.4209
18	Profenofos	0.05	0.043	0.046	0.045	0.0447	0.0015	3.4209
19	Ethion	0.05	0.043	0.042	0.046	0.0437	0.0021	4.7679
20	Trizophos	0.05	0.046	0.044	0.046	0.0453	0.0012	2.5478
21	Edfinphos	0.05	0.043	0.045	0.048	0.0453	0.0025	5.5522
22	Anilophos	0.05	0.046	0.043	0.044	0.0443	0.0015	3.4466
23	Phosalone	0.05	0.046	0.043	0.047	0.0453	0.0021	4.5926

Table 4: Results of individual and combined uncertainties with expanded uncertainty for Organophosphorus pesticides from spiked bottle gourd matrix at 0.05 ppm.

S. No.	Pesticide	Purity %	Wt. std	Uncertainity	SU1	U1	U2	SD-Recovery	Mean-Recovery	U3	U	2U	% uncertainty
1	Dichlorvos	98.5	1.25	0.25	0.1443	0.1465	0.0200	0.0032	0.0437	0.000081	0.0065	0.0129	29.58
2	Monocrotophos	96	1.36	0.25	0.1443	0.1504	0.0184	0.0031	0.0447	0.000080	0.0068	0.0135	30.29
3	Phorate	99.4	1.54	0.25	0.1443	0.1452	0.0162	0.0012	0.0393	0.000027	0.0057	0.0115	29.22
4	Dimetoate	96	1.67	0.25	0.1443	0.1504	0.0150	0.0015	0.0433	0.000037	0.0065	0.0131	30.22
5	Diazinon	98.9	1.32	0.25	0.1443	0.1459	0.0189	0.0031	0.0427	0.000076	0.0063	0.0126	29.43
6	Paraxon-methyl	97.2	1.98	0.25	0.1443	0.1485	0.0126	0.0006	0.0427	0.000015	0.0064	0.0127	29.81
7	Phosphomidon	98.9	1.56	0.25	0.1443	0.1459	0.0160	0.0023	0.0433	0.000057	0.0064	0.0127	29.36
8	Fenthion	99	1.87	0.25	0.1443	0.1458	0.0134	0.0025	0.0433	0.000062	0.0063	0.0127	29.28
9	Chlorpyrifos-methyl	99.7	1.78	0.25	0.1443	0.1448	0.0140	0.001	0.043	0.000025	0.0063	0.0125	29.09
10	Parathion methyl	99.7	1.46	0.25	0.1443	0.1448	0.0171	0.0015	0.0457	0.000040	0.0067	0.0133	29.16
11	Fenitrothion	99.5	1.56	0.25	0.1443	0.1451	0.0160	0.0021	0.0423	0.000051	0.0062	0.0123	29.19
12	Malathion	99.5	1.35	0.25	0.1443	0.1451	0.0185	0.0015	0.0403	0.000035	0.0059	0.0118	29.25
13	Chlorpyrifos	99.3	1.67	0.25	0.1443	0.1454	0.0150	0.0035	0.0427	0.000086	0.0062	0.0125	29.22
14	Parathion	98.8	1.4	0.25	0.1443	0.1461	0.0179	0.003	0.043	0.000074	0.0063	0.0127	29.44
15	Chlorfenvinfos	99.5	1.98	0.25	0.1443	0.1451	0.0126	0.0015	0.0443	0.000038	0.0065	0.0129	29.12
16	Quinolphos	99.3	1.68	0.25	0.1443	0.1454	0.0149	0.0021	0.0427	0.000052	0.0062	0.0125	29.22
17	Fenamiphos	96	1.59	0.25	0.1443	0.1504	0.0157	0.0015	0.0447	0.000039	0.0068	0.0135	30.23
18	Profenofos	99.2	1.67	0.25	0.1443	0.1455	0.0150	0.0015	0.0447	0.000039	0.0065	0.0131	29.25
19	Ethion	98	1.37	0.25	0.1443	0.1473	0.0182	0.0021	0.0437	0.000053	0.0065	0.0130	29.68
20	Trizophos	97.8	1.49	0.25	0.1443	0.1476	0.0168	0.0012	0.0453	0.000031	0.0067	0.0135	29.71
21	Edfinphos	98.5	1.67	0.25	0.1443	0.1465	0.0150	0.0025	0.0453	0.000065	0.0067	0.0133	29.46
22	Anilophos	98.4	1.32	0.25	0.1443	0.1467	0.0189	0.0015	0.0443	0.000038	0.0066	0.0131	29.58
23	Phosalone	98.6	1.37	0.25	0.1443	0.1464	0.0182	0.0021	0.0453	0.000055	0.0067	0.0134	29.50

SU1 = Standard uncertainity of analytical standards

U1 = Relative Standard Uncertainity of analytical standards

U2 = Relative Standard Uncertainity of weighing

U3 = Uncertainity associated with precision

U = Combined Uncertainity

2U = Expanded Uncertainity

Conclusion

Twenty-three organophosphorus pesticides uncertanity estimation of in bottle gourd matrix shows that the values obtained are within permissible limit as per codex and EURACHEM/ CITAC (2000) guidelines. Major sources of uncertainty common to most all methods are considered. As the experiment is performed in well equipped, NABL, and BIS accrediated lab, the data generated is realistic and trustful. So the data can be used for reporting of results and also helps laboratory in extension of analysis of scope.

Aknowlegement

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Declaration

Authors made declaration that the manuscript is our own original work and it does not duplicate any other previously published work. Authors do not have any conflict of interest.

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