WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 6, 1164-1176. Research Article ISSN 2277–7105

UNCERTANITY OF MEASUREMENT DURING ESTIMATION OF 28 PESTICIDES RESIDUE PRESENT IN BOTTLE GOURD

Sudeep Mishra[1], Neelam Richhariya[2]*, Rachana Rani[3], Lalitesh K. Thakur[1], Vikash[1], Vineet Kumar Pandey[1] and Shubham Bansal[1]

[1]Institute of Pesticide Formulation Technology, Min. of Chem. & Fertl. Govt. of India, Sector-20, Udyog Vihar, Gurgaon, Haryana-122016, India.

[2]Govt. Indra College Satna Madhya Pradesh, India.

[3]Banasthali Vidhyapith Jaipur, India.

Article Received on
06 April 2021,

Revised on 27 April 2021,
Accepted on 17 May 2021

DOI: 10.20959/wjpr20216-20564

***Corresponding Author**
Neelam Richhariya
Govt. Indra College Satna
Madhya Pradesh, India.

ABSTRACT

The study was conducted for the calculation of uncertainty in bottle gourd 28 pesticides were taken for study. All the pesticides gave good response to Gas chromatography electron capture detector. For calculation of uncertainty of method, major sources like weighing of standard, purity of certified reference material, precision study i.e repeatability and standard solution preparation were considered. The steps for calculation of uncertainty is identification of uncertainty sources, quantification of uncertainty sources and calculation of combined uncertainty. Combined standard uncertanity of standard preparation due to volumetric flask and micropipette is 0.00092 and calculation due to purity of certified reference material lies between 0.00289-0.0030 and uncertainty due to precision lies between 0.0006 to 0.0025. From all the individual uncertainty calculated are combined, later converted to expanded uncertainty. Percent uncertainty lies between 14 to17 percent which is with in permissible as per EURACHEM/CITAC 2000.

KEYWORDS: Chilli, Uncertanity, Combined Uncertanity, Expanded Uncertanity, Pesticide

INRODUCTION

Guideline for good laboratory practices in residue analysis have been developed on CAC (2003).[1] Method validation and analytical quality control requirements assist laboratories producing reliable and reproducible analytical results.[2] ISO/IEC 17025 accrediated laboratories make available the uncertainty associated with analytical results.[3] To calculate

uncertainty, measurand is firstly specified, possible sources of uncertainty are identified, uncertainty components are quantified, and finally combined uncertainty is calculated.[4] For estimating the uncertainty of 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. The bottom-up approach is very laborious and needs specific knowledge of the whole procedure. The contribution of the individual procedures or steps to the overall uncertainty of the results, are optimized to fit for the purpose of the analysis with minimum cost.[5] The top-down method is dependent on the results of inter-laboratory proficiency tests, collaborative trials, internal quality control data, and inter- or intra-laboratory validation studies (precision and trueness). The second edition of EURACHEM Guide uses the validation and related data for obtaining uncertainty estimates.[4] Alder *et al.,* (2001) estimated between laboratories' relative reproducibility standard deviation of 25% for pesticide residue analysis from proficiency test results in the concentration range of 1 µg/kg–10 mg/kg.[6] There are some more standard and guidelines, based on top-down method.[7-9] Codex Committee on pesticide residue is in the step of proposing a revised guideline on the uncertainty estimation of pesticide residue analysis from simplified top-down approach.[10] 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.[11]

II-EXPERIMENTAL SECTION

2.1. Solvents, Chemicals and Reagents

Chemicals like Florisil, anhydrous sodium sulphate, sodium chloride, glass wool, celite 545, charcoal, magnesium oxide, cotton, filter paper, and magnesium sulphate anhydrous were used as solvents like acetone, acetonitrile, ethyl acetate, methanol, and n-hexane, all of them were purchased from Merck Germany. Primary Secondary Amine i.e. PSA (40 µm, Bondesil) and C-18 silica sorbent were purchased from Sigma Aldrich. The use of high purity reagents and solvents help to minimize interference problems. Bottle gourd fruit taken for study remains free of pesticides.

2.2. Standard Preparation

Certified Reference Standards of known purity were procured from Sigma Aldrich. Total 28 pesticides under study were (alpha-HCH, beta-HCH, gamma HCH, delta HCH, Alachlor,

Aldrin, Dicofol, Pendimethlin, o,p DDE, alpha-Endosulphan, Heptachlor, p,p DDE, Endosulphan Sulphate, Dialdrin, o,p DDD, beta- Endosulphan, p,p DDD, o,p DDT, p,p DDT, Bifenthrin, Fenpropathrin, Lambda Cyhalothrin, beta Cyfluthrin, Cypermethrin, Fenvalarate, Fluvalinate and Deltamethrin). The selection on pesticides based on their common use in agriculture. Standard stock solution of about 100 ppm were prepared. Standards were weighed directly in volumetric flask of 10 ml on analytical balance (Mettler, Toledo). Standard stock solutions were diluted to prepare working secondary standard solutions at, 0.005, 0.01, 0.05, 0.10, 0.50, 1.00 mg/kg concentration. Prepared standards were checked for their response to Gas chromatography with Electron Capture Detector (ECD). All pesticides taken for study give good resposnse to ECD. Calibration and recovery study were done using these working standard solutions of a mixture of pesticides.

III-ANALYTICAL METHODS

3.1 Extraction and Clean up

QuEChERS (quick, easy, cheap, effective, rugged and safe) method was used for extraction.[12] Bottle gourd fruit was finely chopped and homogenized in a mixer grinder. Fifteen gram of homogenized sample was weighed and extracted using 30 ml ethylacetate. Ten gram anhydrous Na_2SO_4 was added, shaken and centrifuged about 6,000 rpm at about 5 minutes. Cleanup of samples using Pimary Secondary Amine (PSA), Magnesium Sulphate anhydrous and activated charcoal.[13] 6 ml extract was cleaned using 0.9 g anhydrous MgSO4, 0.25 g PSA and 0.25 g activated charcoal to remove pigments were added and shaken for 1 min, were centrifuged at 6,000 rpm for 5 min. The upper aliquot 4 ml was dried and later reconstituted by adding 1 ml n-hexane. The reconstituted sample was analysed by Gas Chromatography (GC) Electron Capture Detector.

3.2 Gas Chromatography – Electron Capture Detector (GC-ECD)

GC Agilent make, model 7890B (7693 auto sampler) was used for analysis. DB-5MS fused silica capillary column (30 meter × 0.25 mm, film thickness 0.25µm) was used for separation of pesticides taken for study. GC oven temperature was 170°C as initial for 5 min which increases by a ramp rate of 2°C/min up to 210°C for 5 min., 1°C/min up to 215°C for 5 min. and 4°C/min. up to final temperature of 280°C with a hold time of 8 min. The injector was at 250°C in splitless mode and detector temperature at 300°C. Injection volume 1.0 micro litre, makeup flow 25ml/min., septum purge flow 3 ml/min and equilibrium time 1 min. Total flow

63.75 ml/min with average velocity 18.725 cm/sec and pressure 6.582 psi. Nitrogen was used as makeup gas and helium as carrier gas at a flow rate of 0.75 mL/min.

3.3 Determination of Uncertanities

3. 3.1 Theoretical aspects of uncertainty estimation

Uncertainty estimation steps involve measurand specificity, identity of uncertainty sources, quantification of uncertainty sources calculated depending on standard deviation value which can be directly used, declared uncertain value obtained from calibration certificate and confidence level. Similarly, standard uncertainty (u(x)), expressed as a standard deviation, and expanded uncertainty (U(x)) which is calculated from a combined standard uncertainty and a coverage factor k. Some time uncertainty value is normalised by calculating relative standard uncertainty (u rel) which obtained as the quotient between the standard uncertainty u(x) and the value of x

Urel(x) = U(x)/x

or urel(x) = u(x)/x

Combined uncertainty calculations.

All different contributions of uncertainty have to be combined according to the appropriate rules for giving a combined standard uncertainty:

u = square root of ((x∗ x) + (y∗ y)+· ·)

Applying the appropriate coverage factor, the expanded uncertainty will be obtained.

3.3.2 Uncertanities During Validation of Quantitative Chromatography Method

EURACHEM/CITAC guidelines are followed for calculation of measurement uncertainty of 28 pesticides in bottle gourd. Uncertanity occurs during standard solution preparation. Uncertanity during standard depends upon purity of standards, weight of certified reference standard taken, volumetric flask used for standard preparation and volume measured by micropipette. Uncertanity arise during calibration curve plotting, sampling, sample preparation, repeatability of results, recovery percentage, purity of CRM and Gas Chromatography responses. During sample preparation uncertainty due to weighing can be taken into consideration.

IV. RESULTS AND DISCUSSIONS

The aim of this study was to estimate uncertainty arise during method validation and analysis of 28 pesticides residues in bottle gourd. The steps involved in uncertainty are:

(i) identification of uncertainty sources.

(ii) quantification of uncertainty sources.

(iii)calculation of the combined standard uncertainty.

The uncertainty in each step consists of random and systematic error, which was quantified and included in the combined standard uncertainty. In multi-residue method many sources of uncertainty arise due to gravimetric and volumetric steps (sample weighing, dilution of sample extracts, uncertainty of volume of GPC loop, evaporation of sample extracts, temperature, etc.) which contribute to the overall uncertainty. Major uncertainty arises mainly due to purity of CRM, weighing, volumetric flask, micro pipette and repeatability of results. Uncertainty is very important step for method development process. As per the statistical procedure of the EURACHEM/CITAC Guide CG 4.[4] Combined uncertainty (U) was determined at 0.05 mg/kg level for all the pesticides taken under study.

4.1 Identification of Uncertainty Sources

4.1.1Repeatability

4.1.2 Recovery

4.1.3 CRM purity

4.1.4 Weighing

4.1.5 Preparation of std. solution

4.1.6 GC response

4.1.7 Sample homogeneity

4.2 Quantification of Uncertainty Sources

a. Calibrated, class A glasswares (10ml) volumetric flask were used, so uncertainty due to glasswares is ±0.01ml.

b. Calibrated micro pipettes of 1000 was used, for dilution of standard solution to various concentration levels, so uncertainty due to calibrated micro pipettes are ±0.001.

c. During precision study, linearity of response of GC response has been included, hence separate calculation is not necessary.

d. The estimation of uncertainty for sample homogeneity is complicated and impractical, so neglected.

4.3 Major Uncertanity Sources

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

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

4.3.3 Uncertainty in preparation of std. solution (U3)

a. Volumetric flask (10ml).

b. Micro pipette (1ml).

4.3.4 Uncertainty associated with precision (U4) i.e repeatability.

4.3.1 Calculation of uncertainty by purity of analytical standards (U1)

All 28 pesticides taken for study have their specific purity percent which is mentioned in the certificate of analysis. For calculating standard uncertainty, from uncertainty value given in the certificate, rectangular distribution is applied in which uncertainty value u(x) is divided by √3. So the formula is-. SU1 = (u (x) /√3). From uncertainty table 1, uncertainty of all pesticides CRM purity are almost same i.e 0.5% which is converted to (0.005).

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

S.NO.	Pesticide Standard	Purity of Standard	Uncertainty of Standard (0.05%)	Standard Uncertainty (SU1)	Relative Standard Uncertainty (U1)
1	Alpha-HCH	99.6	0.005	0.0028868	0.0028983
2	Dicofol	99.5	0.005	0.0028868	0.0029013
3	Beta-HCH	99.8	0.005	0.0028868	0.0028925
4	Gamma HCH	99.6	0.005	0.0028868	0.0028983
5	Delta HCH	99.7	0.005	0.0028868	0.0028954
6	Heptachlor	98.2	0.005	0.0028868	0.0029397
7	Alachlor	99.8	0.005	0.0028868	0.0028925
8	Aldrin	99.2	0.005	0.0028868	0.0029100
9	Pendimethalin	99.8	0.005	0.0028868	0.0028925
10	O,P DDE	99.4	0.005	0.0028868	0.0029042
11	Alpha-Endosulphan	99.5	0.005	0.0028868	0.0029013
12	Butachlor	99.3	0.005	0.0028868	0.0029071
13	Dialdrin	99	0.005	0.0028868	0.0029159
14	P,P DDE	99.4	0.005	0.0028868	0.0029042
15	O,P DDD	99.7	0.005	0.0028868	0.0028954
16	P,P DDT	96	0.005	0.0028868	0.003007
17	Beta- Endosulphan	99.5	0.005	0.0028868	0.0029013
18	P,P DDD	96	0.005	0.0028868	0.003007
19	O,P DDT	99.6	0.005	0.0028868	0.0028983
20	Endosulphan Sulphate	99	0.005	0.0028868	0.0029159
21	Bifenthrin	99.5	0.005	0.0028868	0.0029013
22	Fenpropathrin	99.5	0.005	0.0028868	0.0029013
23	Lambda Cyhalothrin	98.5	0.005	0.0028868	0.0029307
24	Beta Cyfluthrin	99.5	0.005	0.0028868	0.0029013
25	Cypermethrin	99.5	0.005	0.0028868	0.0029013
26	Fenvalarate	99.3	0.005	0.0028868	0.0029071
27	Fluvalinate	99.8	0.005	0.0028868	0.0028925
28	Deltamethrin	99.5	0.005	0.0028868	0.0029013

4.3.2 Calculation of uncertainty of weighing (U2)

1-2 mg of weight is taken during weighing of Certified Reference Material. The uncertainty value i.e 0.001gm of the weighing balance is considered in which normal distribution of weight is taken. Standard uncertainty is calculated by the equation = 0.0001/2, whereas relative standard uncertainty U2=(0.0001/2)/W, whereas W is the weight of pest 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. The calculation of uncertainty due to weighing of certified reference standards are shown in Tabe-2.

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	Alpha-HCH	1.24	0.0001	5.77E-05	4.66E-05
2	Dicofol	1.91	0.0001	5.77E-05	3.02E-05
3	Beta-HCH	1.37	0.0001	5.77E-05	4.21E-05
4	Gamma HCH	1.86	0.0001	5.77E-05	3.10E-05
5	Delta HCH	1.48	0.0001	5.77E-05	3.90E-05
6	Heptachlor	1.2	0.0001	5.77E-05	4.81E-05
7	Alachlor	1.25	0.0001	5.77E-05	4.62E-05
8	Aldrin	1.23	0.0001	5.77E-05	4.69E-05
9	Pendimethalin	1.73	0.0001	5.77E-05	3.34E-05
10	O,P DDE	1.8	0.0001	5.77E-05	3.21E-05
11	Alpha-Endosulphan	1.45	0.0001	5.77E-05	3.98E-05
12	Butachlor	1.27	0.0001	5.77E-05	4.55E-05
13	Dialdrin	1.56	0.0001	5.77E-05	3.70E-05
14	P,P DDE	1.84	0.0001	5.77E-05	3.14E-05
15	O,P DDD	1.87	0.0001	5.77E-05	3.09E-05
16	P,P DDT	1.82	0.0001	5.77E-05	3.17E-05
17	Beta- Endosulphan	1.57	0.0001	5.77E-05	3.68E-05
18	P,P DDD	1.46	0.0001	5.77E-05	3.95E-05
19	O,P DDT	1.83	0.0001	5.77E-05	3.15E-05
20	Endosulphan Sulphate	1.74	0.0001	5.77E-05	3.32E-05
21	Bifenthrin	1.46	0.0001	5.77E-05	3.95E-05
22	Fenpropathrin	2.1	0.0001	5.77E-05	2.75E-05
23	Lambda Cyhalothrin	1.54	0.0001	5.77E-05	3.75E-05
24	Beta Cyfluthrin	1.36	0.0001	5.77E-05	4.25E-05
25	Cypermethrin	1.45	0.0001	5.77E-05	3.98E-05
26	Fenvalarate	1.89	0.0001	5.77E-05	3.05E-05
27	Fluvalinate	1.87	0.0001	5.77E-05	3.09E-05
28	Deltamethrin	1.56	0.0001	5.77E-05	3.70E-05

4.4.1 Uncertainty in preparation of std. solution (U3)

Uncertanity arise due to standard preparation are.

a. Volumetric flask (10ml). Calibrated, class A glasswares were used, so standard uncertainty at 95% confidence level is 0.001.

b. Fill and weight experiment: Readings were taken to calculate standard uncertainty arise during experiment is 0.00286

c. Temperature Variation: Effect of temperature in the standard preparation at 25 degree Celsius is 0.00242.

d. Micro pipette; calibrated micro-pipettes of 1000 microlitre was used, so standard uncertainty due to micro pipette is 0.001.

Therefore the combined uncertainty value of the entire factor involves in standard preparation is 0.00092.

4.4.3 Uncertainty arises due to precision (U4)

Repeatability of three replicate recovery in which their mean value, standard deviation, relative standard deviation were calculated. Uncertainty arise due to precision of 28 mixture pesticides, are shown in table 3. Errors caused during sample processing steps i.e extraction, clean up, and GC analyses were approximated by standard deviations (s), calculated from triplicate determinations of analytes expressed as repeatability 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: Sources of Uncertainty of standard Preparation.

S.NO.		Confidence level factor	Values on certificate	Readings taken	Standard uncertainty	Combine standard uncertainty
1	Volumetric Flask					
	Certificate value (10ml)	2	0.001	10	0.0001	0.00092
	Fill & weigh experiment	Std dev.	0.0064		0.00063	
	Temperature variation	1.73205	0.0042		0.00042	
2	Micropipette (1ml)	2	0.001	4	0.0005	

UNCERTANITY BUDJET

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, U3 and U4 are taken. The combined uncertainty (U) was

calculated by equation: $U = x [(U1)^2+(U2)^2+(U3)^2+(U4)^2]^{1/2}$. Expanded uncertainty (2U) was twice of combined uncertainty (U) at 95% confidence level. From table no.4, combined uncertainty values lies between 0.0007-0.0035 (table-4). Also percent uncertainty value is calculated by dividing expanded uncertanity value by recovered amount value and which is taken 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 almost all the pesticides taken for study from table was found in the range of (b) 11-15% and (c) 15-20%. Table 4, shows individual uncertainties and combined uncertainties with expanded uncertainty for 28 pesticides from spiked bottle gourd matrix at 0.05 ppm.

Table 4: Results of individual and combined uncertainties with expanded uncertainty for organochlorine, synthetic pyrithroids and herbicides pesticides from spiked bottle gourd matrix at 0.05 ppm.

S. NO.	Pesticide	Mean Recovered	U1	U2	U3	U4	U	2U	Uncertainty	% Uncertainty
1	Alpha-HCH	0.043	0.002898	0.0000466	0.000915	0.0012	0.003268	0.006536	±0.007 of 0.043	15
2	Dicofol	0.044	0.002901	0.0000302	0.000915	0.0015	0.003392	0.006784	±0.007 of 0.044	15
3	Beta-HCH	0.046	0.002893	0.0000421	0.000915	0.0017	0.003478	0.006956	±0.007 of 0.046	15
4	Gamma HCH	0.042	0.002898	0.000031	0.000915	0.0014	0.003346	0.006693	±0.007 of 0.042	16
5	Delta HCH	0.044	0.002895	0.000039	0.000915	0.0009	0.003167	0.006335	±0.006 of 0.044	14
6	Heptachlor	0.045	0.00294	0.0000481	0.000915	0.0003	0.003094	0.006188	±0.006 of 0.045	14
7	Alachlor	0.043	0.002893	0.0000462	0.000915	0.0009	0.003165	0.00633	±0.006 of 0.043	15
8	Aldrin	0.043	0.00291	0.0000469	0.000915	0.0006	0.003109	0.006219	±0.006 of 0.043	14
9	Pendimethlin	0.043	0.002893	0.0000334	0.000915	0.0014	0.003341	0.006683	±0.007 of 0.043	16
10	O,P DDE	0.044	0.002904	0.0000321	0.000915	0.0006	0.003104	0.006207	±0.006 of 0.044	14
11	Alpha-Endosulphan	0.043	0.002901	0.0000398	0.000915	0.0006	0.003101	0.006202	±0.006 of 0.043	14
12	Butachlor	0.044	0.002907	0.0000455	0.000915	0.0012	0.003276	0.006551	±0.007 of 0.044	15
13	Dialdrin	0.043	0.002916	0.000037	0.000915	0.0021	0.003708	0.007416	±0.007 of 0.043	17
14	P,P DDE	0.042	0.002904	0.0000314	0.000915	0.0012	0.003273	0.006546	±0.007 of 0.042	16
15	O,P DDD	0.042	0.002895	0.0000309	0.000915	0.0025	0.003933	0.007867	±0.008 of 0.042	19
16	P,P DDT	0.041	0.003007	0.0000317	0.000915	0.0017	0.003574	0.007147	±0.007 of 0.041	17
17	Beta- Endosulphan	0.043	0.002901	0.0000368	0.000915	0.0009	0.003173	0.006345	0.007 of 0.043	15
18	P,P DDD	0.041	0.003007	0.0000395	0.000915	0.0014	0.003441	0.006882	0.007 of 0.041	17
19	O,P DDT	0.043	0.002898	0.0000315	0.000915	0.0012	0.003268	0.006536	0.007 of 0.043	15
20	Endosulphan Sulphate	0.044	0.002916	0.0000332	0.000915	0.0012	0.003283	0.006567	0.007 of 0.044	15
21	Bifenthrin	0.045	0.002901	0.0000395	0.000915	0.0014	0.003349	0.006698	0.007 of 0.045	15
22	Fenpropathrin	0.045	0.002901	0.0000275	0.000915	0.0006	0.003101	0.006202	0.006 of 0.045	14
23	Lambda Cyhalothrin	0.042	0.002931	0.0000375	0.000915	0.0017	0.00351	0.007019	0.007 of 0.042	17
24	Beta Cyfluthrin	0.044	0.002901	0.0000425	0.000915	0.0009	0.003173	0.006346	0.006 of 0.044	14

25	Cypermethrin	0.043	0.002901	0.0000398	0.000915	0.0009	0.003173	0.006346	0.006 of 0.043	15
26	Fenvalarate	0.044	0.002907	0.0000305	0.000915	0.0014	0.003354	0.006708	0.007 of 0.044	15
27	Fluvalinate	0.045	0.002893	0.0000309	0.000915	0.0012	0.003263	0.006525	0.007 of 0.045	15
28	Deltamethrin	0.043	0.002901	0.000037	0.000915	0.0009	0.003173	0.006345	0.006 of 0.043	15

Relative Standard Uncertanity due purity of certified reference standards (U1)

Relative Standard Uncertanity due to weighing (U2)

Combined Standard uncertainty of volumetric flask & micropipette (U3)

Standard Uncertainty of Precision (U4)

CONCLUSION

Uncertainty estimation of method for 28 pesticides 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 almost 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

Authors are thankful to Director IPFT for encouraging and providing facilities for research work.

REFERENCES

1. Codex Alimentarius Commission (CAC), Guidelines on good laboratory practice in residue analysis. FAO, Rome; CAC/GL 40–1993: Revision 1 2003.
2. SANCO (Directorate General for Health and Consumer Affairs), Method validation and quality control procedures for pesticide residues analysis in food and feed. SANCO 12495/2011.
3. Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation International Standard Organization and International Electrochemical Community ISO/IEC 170205:2005, General requirements for the competence of testing and calibration laboratories (ISO/IEC), 2005.
4. Guide quantifying uncertainty in analytical measurements. 2nd edition; EURACHEM/CITAC, 2000.
5. Ambrus A. Reliability of measurements of pesticide residues in foods. Accred Qual Assur, 2004; 9(6): 288-304.
6. Alder L, Korth W, Patey AL, Van Der Schee HA, Schoeneneweiss S. Estimation of measurement uncertainty in pesticide residue analysis. J AOAC Int, 2001; 84: 1569-1578.
7. Barwick VJ, Ellison SLR. Development and harmonization of uncertainty principles, Part (d): Protocol for uncertainty evaluation from validation data. LGC/Valid Analytical Measurement; Teddington, 2000.
8. International Standard Organization (ISO) (1993). Guide to the expression of uncertainty in measurement, International Organization for Standardization (ISO), Geneva International Standard Organization (ISO), (2004); ISO/TS 21748: 2004.

9. Magnusson B, Naykki T, Hovind H, Krysell M. Handbook for calculation of measurement uncertainty in environmental laboratories, 2nd Edition. NORDTEST Report TR537, Nordic Innovation Center; Finland, 2004.
10. Codex Alimentarius Commission (CAC), Report of the fortyfirst session of the Codex Committee on Pesticide Residues. FAO, Rome, ALINORM 09/32/24, Appendix X (2009b).
11. Codex Alimentarious Commision (CAC), Report of Thirthy- First Session of Codex Committee on Methods of Analysis and Sampling (CCMAS); ALINORM 10/33/23; 2010.
12. Anastassiades M, Lehotay SJ., Stajnbaher D. & FJ. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. J of AOAC Inter, 2003; 86: 412-431.
13. Lehotay SJ. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: Collaborative study. J of AOAC Inter, 2007; 90(2): 485-520.