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Uncertanity Estimation of 28 Pesticides Residues in Chilli by Gas Chromatography, Electron Capture Detector (GC-ECD)

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Abstract

The study was undertaken to calculate the uncertainty of results, related to the analysis of 28 pesticides present in green chilli. Gas Chromatography Electron Capture Detector was used for the analysis of above pesticides present in green chilli. Bottom-up approach was taken for calculation of uncertainty sources arise from weighing, purity of standards, repeatability, calibration and recovery study. To calculate the total uncertainty, relative uncertainty due to purity of standard (U1), due to weighing (U2) and precision (U3) are considered. The combined uncertainty (U) was calculated by equation: $U = [(U1)2+(U2)2+(U3)2]^{1/2}$. Expanded uncertainty (2U) was twice of combined uncertainty (U) at 95% confidence level. Combined uncertainty values lies between 0.0007-0.0035. Percent uncertainty of almost all the pesticides taken for study was found below ≤10% except beta HCH percent uncertainty value is 11% lies in 11-15% range and lambdacyhalothrin value is 16% lies in 15-20% range.

Keywords: Chilli; Uncertanity; Combined Uncertanity; Expanded Uncertanity; Pesticide.

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Introduction

Uncertanity arise either random or as systematic errors which give information about the range of results expected. Uncertanity can be estimated by analytical method of detailed operating procedure. Definition of uncertainty of measurement is a parameter associated with the measurement and dispersion of values attributed to the measurand.[1] The estimation and evaluation of an uncertainty associated-with the result of chemical analysis can be found in SR ENV 13005:2003 guide and in the Eurachem/CITAC Guide CG4. As per SR EN ISO/CEI 17025:2005 all certified laboratories must apply the procedures for the estimation of the uncertainty of the measurement. Bottomup[2], top down and inhouse validation are proposed for expression of uncertainty.[3] Pesticide residue laboratory use bottom-up approach in conjuction with in-house validation[4,5] data for estimating the uncertainty derived from each step of the analytical method.[6-8]

By various analytical steps during the experiment, uncertainty originates from many sources such as sampling matrix effect, uncertainty due to masses, volumetric equipments, reference standards, approximation and assumption are incorporated in the method. Uncertainty of each analytical step consists of its random and systematic error which are qualified and incorporated into the combined standard uncertainty.

This paper based on methodology for estimating the uncertainty associated to multiresidue analytical method in chilli matrix, through the bottom-up

approach and on the basis of in-house validation data.

Materials and Methods

Solvents, Chemicals and Reagents

HPLC grade solvents like. 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. The use of high purity reagents and solvents help to minimize interference problems. Chilli fruit free of pesticides were obtained from organic farms of Satna district of Madhya Pradesh, India.

Standard Preparation

Pesticide standards were of high purity above 98% were procured from Sigma Aldrich. 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). These pesticides are commonly used by farmers of India. CRM of individual pesticide was weighed directly in volumetric flask of 10 ml. on analytical balance (Mettler, Toledo) and dissolved in few drops of HPLC grade acetone which was further reconstituted with HPLC grade n-hexane. Secondary Standard solutions were prepared at, 0.005, 0.01, 0.05, 0.10, 0.50, 1.00 mg/kg which gives good resposnse for Electron Capture Detector of Gas Chromatography. All these working standard solutions of a mixture of pesticides were prepared for calibration and recovery tests.

Extraction and Clean up

QuEChERS (quick, easy, cheap, effective, rugged and safe) method[9] was used for extraction of chilli sample with some modifications. The steps involved are: chilli fruit was finely chopped and homogenized in a mixer grinder. Fifteen gram of homogenized sample weighed into a 50 ml centrifugation tube and 30 ml of Ethyl acetate was added and shaken for 1 min. Ten gram anhydrous Na_2SO_4 was added and shaken. The tube was centrifuged at 6,000 rpm at about 5 minutes. Cleanup was performed according to Lehotay (2007).[10] 6 ml extract was transferred from the upper layer into a 15 ml centrifuge 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 6,000 rpm for 5 min. The supernatant 4 ml was dried in turbovap. The dried sample was reconstituted by adding 1 ml n-hexane. The reconstituted sample was used for GC analysis.

Gas Chromatography – Electron Capture Detector (GC-ECD)

Agilent 7890B (7693 auto sampler) equipped with DB-5MS capillary column (30 meter × 0.25 mm, film thickness 0.25µm) fused silica capillary column was used for preliminary screening and final quantification of pesticide residues. Oven temperature programming was 170°C as initial temperature for 5 min followed 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 (splitless mode) and detector temperature were set at 250°C, 300°C, respectively. 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.

Determination of Uncertanities

Theoretical aspects of uncertainty estimation

Expressing uncertainty in way different way, 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. In some cases, it is feasible to use relative uncertainties (in both uncertainties), which represent the value of the uncertainty normalised. It is obtained as the quotient between the standard uncertainty u(x) and the value of x:

$Urel(x) = U(x)/x$

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

The steps involved in uncertainty estimation are as follows.

- To Specify the measurand.

- Relationship between the measurand and the input quantities, such as measured quantities, constants and calibration standard values.
- Identify uncertainty sources. Specified uncertainty sources in the above step.
- Quantify uncertainty components. Associated with each potential source of uncertainty identified.
  - The different contributions to the overall uncertainty can be calculated depending on the data available:
  - from a standard deviation value: this value is directly used;
  - from the standard deviation of experimental data sets;
  - from a declared uncertainty value, which is given in a certificate of calibration;
  - from a confidence interval;
  - from a range of limits(upper and lower limits);
  - finally from a given error value.
- Calculate combined uncertainty. The different contributions to the overall 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.

Determination of Uncertanities During Validation of Quantitative chromatography

Method

The measurement uncertainty was calculated as per EURACHEM/CITAC and quantifying uncertainty for 28 pesticides residue 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

Results and Discussions

Uncertainty arise during method validation and analysis of 28 pesticides residues in chilli. The aim of this study was to estimate uncertainties involved in analysis of 28 pesticides residues in chilli involved following steps:

1. identification of of uncertainty sources.
2. quantification of uncertainty sources.
3. calculation of the combined standard uncertainty.

The uncertainty of each individual analytical step consists of its random and systematic component which of these was quantified and incorporated in the combined standard uncertainty. There are many potential sources of uncertainty described in multi-residue methods includes all 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. However, detailed exploration and evaluation of all these uncertainty sources is complicated and impractical.

Therefore it is important 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[1].

- Identification of Uncertainty Sources
- Repeatability
- Recovery
- Uncertainty in CRM purity
- Uncertanity in weighing
- Uncertainty in preparation of std. solution
- Uncertainty in GC response

- Uncertainty in sample homogeneity

Measur and

ppm conc. = area of sample X conc. of standard X dilution X 1 area of standard sample weight

Quantification of Uncertainty Sources

a. Volumetric flask (10ml). Calibrated, class A glasswares were used, so uncertainty due to glasswares can be neglected.
b. Micro pipette; calibrated pipettes of 1000 and 200 micro litre were used, so uncertainty due to micro pipette can be neglected.
c. GC response; Uncertainty in linearity of response is in given concentration range has been included in the precision study hence separate calculation is not necessary.
d. 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.

Main cause of uncertainty

1. First relative standard uncertainty (U1) due to purity of analytical standards.
2. Uncertainty due to weighing (U2) of analytical CRM.
3. Uncertainty associated with precision (U3) i.e repeatability.

Uncertainty by purity of analytical standards (U1)

From all 28 pesticides with their specific purity percent have uncertainty mentioned in the certificate of purity. Rectangular distribution was 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.

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

S.No.	Pesticide Standard	Purity of Standard	Uncertanity of Standard (0.05%)	Standard Uncertanity (SU1)	Relative Standard Uncertanity (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	Pendimethlin	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

Uncertainty of weighing (U2)

The uncertainty arise during weighing of neat standards. Weight of standards were taken between 1-2 mg. The uncertainty value of the weighing balane is 0.001gm. The normal distribution of weight is taken under consideration. Standard uncertainty due to weighing 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. Considering normal distribution, the uncertainty of the balance was divided by taking two. The calculation of uncertainty due to weighing of certified reference standards are shown in Tabe 2.

Uncertainty arise due to precision (U3)

Uncertainty arise due to precision are shown in table 3. Table shows that for test mixture of 28 mixture pesticides, three replicate recovery and their mean value, standard deviation, relative standard deviation were calculated. , 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/(√n × 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.

Uncertanity Budget

To calculate the total uncertainty, Relative uncertainty due to purity of standard (U1), due to weighing (U2) and precision (U3) 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 0.0007-0.0035. Also percent uncertainty value is calculated by dividing expanded uncertanity value by recovered amount value and multiplied by 100. From the table 4. The expanded uncertainty of the

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

S.No.	Pesticide Standard	Weight of Standard	Uncertanity in Weighing	Standard Uncertanity	Relative Standard Uncertanity (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	Pendimethlin	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

Table 3: Shows Recovery, Mean Recovery, Standard Deviation (S.D), Relative Standard Deviation (RSD) of organochlorine, synthetic pyrithroids and herbicides pesticides from spiked chilli matrix at 0.05 ppm.

S. No.	PESTICIDE	RT	Spiking conc (PPM)	Amount recovered R1	Amount recovered R2	Amount recovered R3	Mean Rec. Amount	Standard Deviation	Standard Uncertainty	Relative Standard Uncertainty (U3)
1	Alpha-HCH	9.8	0.05	0.041	0.043	0.046	0.043	0.0025	0.0015	0.03379
2	Dicofol	10.8	0.05	0.046	0.043	0.042	0.044	0.0021	0.0012	0.027315
3	Beta-HCH	11.25	0.05	0.041	0.049	0.048	0.046	0.0044	0.0025	0.054709
4	Gamma HCH	11.57	0.05	0.046	0.04	0.041	0.042	0.0032	0.0019	0.044189
5	Delta HCH	12.86	0.05	0.041	0.046	0.045	0.044	0.0026	0.0015	0.034716
6	Heptachlor	15.78	0.05	0.046	0.043	0.045	0.045	0.0015	0.0009	0.019598
7	Alachlor	15.87	0.05	0.045	0.043	0.042	0.043	0.0015	0.0009	0.02051
8	Aldrin	18.05	0.05	0.044	0.04	0.046	0.043	0.0031	0.0018	0.041019
9	Pendimethlin	21.06	0.05	0.045	0.042	0.043	0.043	0.0015	0.0009	0.02051
10	O,P DDE	23.21	0.05	0.045	0.042	0.045	0.044	0.0017	0.0010	0.022727
11	Alpha-Endosulphan	23.52	0.05	0.044	0.042	0.043	0.043	0.0010	0.0006	0.013427
12	Butachlor	24.22	0.05	0.045	0.044	0.043	0.044	0.0010	0.0006	0.013122
13	Dialdrin	25.53	0.05	0.041	0.042	0.046	0.043	0.0026	0.0015	0.035524
14	P,P DDE	25.68	0.05	0.045	0.042	0.039	0.042	0.0030	0.0017	0.041239
15	O,P DDD	26.3	0.05	0.045	0.039	0.043	0.042	0.0031	0.0018	0.041996
16	P,P DDT	26.4	0.05	0.039	0.042	0.043	0.041	0.0021	0.0012	0.029313
17	Beta- Endosulphan	28.14	0.05	0.043	0.041	0.044	0.043	0.0015	0.0009	0.02051
18	P,P DDD	29.47	0.05	0.04	0.042	0.042	0.041	0.0012	0.0007	0.01626
19	O,P DDT	29.72	0.05	0.046	0.042	0.041	0.043	0.0026	0.0015	0.035524
20	Endosulphan Sulphate	32.6	0.05	0.044	0.043	0.046	0.044	0.0015	0.0009	0.020044
21	Bifenthrin	41.72	0.05	0.045	0.043	0.046	0.045	0.0015	0.0009	0.019598
22	Fenpropathrin	42.19	0.05	0.045	0.043	0.046	0.045	0.0015	0.0009	0.019598
23	Lambda Cyhalothrin	47.44	0.05	0.042	0.043	0.042	0.042	0.0006	0.0003	0.007937
24	Beta Cyfluthrin	52.7-52.9	0.05	0.044	0.046	0.043	0.044	0.0015	0.0009	0.020044
25	Cypermethrin	53.03-53.44	0.05	0.046	0.041	0.042	0.043	0.0026	0.0015	0.035524
26	Fenvalarate	56.23	0.05	0.044	0.043	0.046	0.044	0.0015	0.0009	0.020044
27	Fluvalinate	56.9-57.2	0.05	0.043	0.045	0.046	0.045	0.0015	0.0009	0.019598
28	Deltamethrin	58.63	0.05	0.046	0.041	0.043	0.043	0.0025	0.0015	0.03379

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

S. No.	Pesticide	Mean Recovered	U1	U2	U3	U	2U	Uncertainty	Percent Uncertainty
1	Alpha-HCH	0.043	0.0028983	4.66E-05	0.03379	0.0014	0.0029	±0.003 of 0.043	7
2	Dicofol	0.044	0.0029013	3.02E-05	0.027315	0.0012	0.0024	±0.002 of 0.044	5
3	Beta-HCH	0.046	0.0028925	4.21E-05	0.054709	0.0025	0.0051	±0.005 of 0.046	11
4	Gamma HCH	0.042	0.0028983	3.10E-05	0.044189	0.0019	0.0037	±0.004 of 0.042	9
5	Delta HCH	0.044	0.0028954	3.90E-05	0.034716	0.0015	0.0030	±0.003 of 0.044	7
6	Heptachlor	0.045	0.0029397	4.81E-05	0.019598	0.0009	0.0018	±0.002 of 0.045	4
7	Alachlor	0.043	0.0028925	4.62E-05	0.02051	0.0009	0.0017	±0.002 of 0.043	4
8	Aldrin	0.043	0.00291	4.69E-05	0.041019	0.0018	0.0036	±0.004 of 0.043	8
9	Pendimethlin	0.043	0.0028925	3.34E-05	0.02051	0.0009	0.0017	±0.002 of 0.043	4
10	O,P DDE	0.044	0.0029042	3.21E-05	0.022727	0.0010	0.0020	±0.002 of 0.044	5
11	Alpha-Endosulphan	0.043	0.0029013	3.98E-05	0.013427	0.0006	0.0012	±0.002 of 0.043	3
12	Butachlor	0.044	0.0029071	4.55E-05	0.013122	0.0006	0.0012	±0.002 of 0.044	3
13	Dialdrin	0.043	0.0029159	3.70E-05	0.035524	0.0015	0.0030	±0.003 of 0.043	7
14	P,P DDE	0.042	0.0029042	3.14E-05	0.041239	0.0017	0.0035	±0.004 of 0.042	8
15	O,P DDD	0.042	0.0028954	3.09E-05	0.041996	0.0018	0.0036	±0.004 of 0.042	9
16	P,P DDT	0.041	0.003007	3.17E-05	0.029313	0.0012	0.0024	0.003 of 0.041	6
17	Beta- Endosulphan	0.043	0.0029013	3.68E-05	0.02051	0.0009	0.0017	0.002 of 0.043	4
18	P,P DDD	0.041	0.003007	3.95E-05	0.01626	0.0007	0.0014	0.002 of 0.041	3
19	O,P DDT	0.043	0.0028983	3.15E-05	0.035524	0.0015	0.0030	0.003 of 0.043	7
20	Endosulphan Sulphate	0.044	0.0029159	3.32E-05	0.020044	0.0009	0.0018	0.002 of 0.044	4
21	Bifenthrin	0.045	0.0029013	3.95E-05	0.019598	0.0009	0.0018	0.002 of 0.045	4
22	Fenpropathrin	0.045	0.0029013	2.75E-05	0.019598	0.0009	0.0018	0.002 of 0.045	4
23	Lambda Cyhalothrin	0.042	0.0029307	3.75E-05	0.007937	0.0035	0.0069	0.007 of 0.042	16
24	Beta Cyfluthrin	0.044	0.0029013	4.25E-05	0.020044	0.0009	0.0018	0.002 of 0.044	4
25	Cypermethrin	0.043	0.0029013	3.98E-05	0.035524	0.0015	0.0030	0.003 of 0.043	7
26	Fenvalarate	0.044	0.0029071	3.05E-05	0.020044	0.0009	0.0018	0.002 of 0.044	4
27	Fluvalinate	0.045	0.0028925	3.09E-05	0.019598	0.0009	0.0018	0.002 of 0.045	4
28	Deltamethrin	0.043	0.0029013	3.70E-05	0.03379	0.0014	0.0029	0.003 of 0.043	7

U1 = Relative Standard Uncertainity of analytical standards; U2 = Relative Standard Uncertainity of weighing; U3 = Uncertainity associated with precision; U = Combined Uncertainity; 2U = Expanded Uncertainity

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 was found below ≤10% lies (a) range except beta HCH percent uncertainty value is 11% lies in (b) range and lambdacyhalothrin value is 16% lies (c) range. Table 4. shows individual uncertanities and combined uncertainties with expanded uncertainty for 28 pesticides from spiked chilli matrix at 0.05 ppm.

Conclusion

The method followed for all pesticides taken for study is efficient in determining of uncertainty of 28 pesticides from chilli matrix. Uncertanity value is calculated for each major step of method validation. Uncertanity arise by various steps of the method are rectified and calculated according to SANCO guidelines.

Aknowlegement

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