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Estimation of uncertainty and variability for sample processing in brinjal (Eggplant) for pesticide residue analysis using organophosphate pesticide ^{14}c-chlorpyrifos

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Abstract

Determination of true values for pesticide residues in food commodities ensures the credibility of the protocol followed in an analytical laboratory. Various proposed protocols for residue analysis fail to monitor the MRLs fixed by governmental regulatory agencies. The central inertia behind such outcomes lies in the undefined methodology followed, where the nature of the matrix, its way of processing, storage, and analytical approach is never well-thought-off. In the present study, for the presence of spiked ^{14}C-labeled organophosphate pesticide Chlorpyrifos the pretreated brinjal extracts were analyzed on Beckman's liquid scintillation counter. This highly sensitive technique to detect minuscule loss of pesticide during sample processing is preferred and recommended for many residue analysis laboratories. The extract obtained after sample processing steps viz. chopping, fine blending, etc., contained the analyte and pigment. These co-extracts would not affect the analysis, as an additional cleanup step removed them. The propensity of pesticide residues loss increments as the number of steps increases, but the additional cleanup step to remove co-extracts did not influence the results. In the current context, diminutive loss of radioactivity at any step is directly proportional to pesticide loss; therefore, after the conclusion of each step, the radioactivity left in the glassware or container used was thoroughly measured and were taken into account for calculating the final residue levels. Homogeneity, statistically denoted as sampling constant (KS value), of the analyte is another very significant factor that entails the certainty of pesticides residues protocol. The sampling constants and combined uncertainty with the Brinjal matrix carried almost similar values, whereas the percentage recovery and reproducibility values calculated showed remarkable distinctions in two processing conditions.

Keywords: sample processing, percentage recovery, µCi (microcurie), CV $_{\text{COMB. SP\%}}$

Introduction

Method validation for pesticide residue analysis is an essential phase in determining the permissible limits of the xenobiotics in food commodities (Harris et al., 2000 and Herrman, 2000). To ensure the credibility of analysis, the procedure applied and the statistics involved in quantifying the pesticide residues are of utmost significance (Ellison et al., 1998). The uncertainties caused due to numerous factors dictate the efficiency of analytical methods being followed (Ellison and Barwick, 1998 and Ellison et al., 1998). By deciphering the nature of the error, random or systematic arisen while sampling or sample processing bring possibilities to rectify them partially or completely (Ambrus A. and, Suszter G., 2019).

Complete guidelines (Ellison and, Williams, 2012) such as EU Reference Laboratories for Residues of Pesticides (2006) and EURACHEM/CITAC (2000) issued by pesticide residue monitoring governmental agencies (Thrope and Reynolds, 1996) supports the analytical chemists (Hortwiz, 1979) to follow procedures which are universal (Visi, 2002) and less prone to errors (Dornseiffen and Van Eck, 2000). The overall random errors emerging during each stage of analysis can be mathematically expressed as (Youden, 1967):

$$\mathbf{S_R} = \sqrt{(S_S)^2 + (S_{SP})^2 + (S_A)^2}$$

in the equation, S_S denotes Variance due to sampling the analytical portion, S_{SP} denotes Variance due to sample processing, and S_A denotes Variance due to Analysis. Suszter et al. in 2006, conceptualized another random error (Ambrus, 2020) that causes relative uncertainty of laboratory samples (CV_{Lab}). The following equation gives expression to estimate the uncertainty

$$\mathbf{CV_{Lab}} = \sqrt{(CV_{SP})^2 + (CV_A)^2}$$

in the equation, CV_{SP} denotes random errors of sample processing, and CV_A denotes random errors of Analysis. Further, various well-defined, less-defined, and other undefined factors of uncertainty are required to be addressed for obtaining suitable recoveries (Barwick and Ellison, 1999) as the reproducible results (Lantos et al., 1983). Amongst several proposed approaches in designing the experiments for residual pesticide recoveries the two most accepted include the "Top-down" and "Bottom-up" approaches (Caudros-Rodr'ẏguez et al., 2002). These approaches were well-described and compared as suited for different laboratory conditions (Stepan et al., 2004).

In a method validation research (Holland et al., 2000 and Hoogerbugge & Zoonen, 2000), starting with the first step ie., pesticide treatment or spiking then sampling-out (Hill, 2000) of analytical portions (Hortwiz, 1988 and Bidaoui-El et al., 2000), the mass transfer of matrix from one location to another for successive phases of sample processing (da Silva et al., 2000), till the conclusive phase of experiment "analysis" (Hortwiz, 2000), systematic errors contribute for false outcomes (Maestroni et al., 2000). In many instances common interfering compounds called co-extracts, adversely affect the detection either by showing an enhancement or diminishing effects due to their presence along with the analyte, in the extract (Schenck, 2000 and Egea González, 2002). Working with organophosphorus pesticides, Erney et al. in 1993 discussed this effect on chromatographic analysis. As observed by Maestroni et al. in 2018, in brinjal the same effect was conspicuously evident hence needed an additional cleanup step for nullifying the false-positive results. Suggestive sample processing procedures under differential temperature conditions (Fussell, 2002 and Suman & Singh, 2011) could tend to resolve the issues evolving due to degradation of the analyte. Therefore, analyte stability is also a keystone factor for residue analysis (Hajsˇlova´et al., 1998 and Tiryaki et al., 2008). Additionally, appropriate solvent selection for pesticide extraction plays a significant role in the efficiency of pesticide recovery. To reduce the usage of solvent for extraction to the minimum it is important to consider the polarity of the analyte pesticide (Wan and Wong, 1996). Misappropriation at this level may lead to variability in analytical reports (Suhre, 2000) lower residue recoveries, and lesser reproducibilities (Aysal, 2007).

The use of radioactivity for tracing the pesticide loss during sample processing is another sensitive means to reduce uncertainties and variability in residue analysis (Caron and Benz, 2002) as a minimal amount of radiolabeled pesticide can be detected. Radioactive ^{14}C-labeled pesticides are used in many instances for method validation studies. For testing the efficiency of sample processing and determining the uncertainties arising due to the same Maestroni et al. in 2000, used ^{14}C-Chlorpyrifos. The organophosphate pesticide ^{14}C-Chlorpyrifos was also used to estimate the uncertainties in the residue analysis procedure on Cucumber and Tomatoes (Tiryaki & Baysoyu, 2008 and Tiryaki et al., 2009).

Materials and Methods

Chemicals: ^{14}C-Chlorpyrifos (IAEA), solvents: Acetone and Analytical grade Ethyl acetate (Merck), Sodium hydrogen carbonate ($NaHCO_3$), Sodium sulfate (Na_2SO_4), Cocktail for Liquid scintillation counting (Hydroflour, National Diagnostics; Atlanta; Georgia) and Dry Ice.

Equipment: Chopper available in Laboratory, Warring blender with steel blades (Make: Khera Instruments), Homogenizer Machine, Refrigerated Centrifuge (Make: Sigma), Beckman's Liquid Scintillation counter, Weighing balance (Top load), -80 ºC deep freezer, hot water bath (available in the laboratory).

Glasswares required: Centrifuge tubes (50 mL & 250 mL); Borosilicate glass beaker (500 ml & 1L thick-walled); Measuring cylinders (10 mL & 100mL); Laboratory thermometer; Scintillation vials; Hamilton's micro syringe (500 µl); spoon/ spatula; aluminium foil; pipette (1 mL); Whatman's filter papers (8 cm diameter), separating funnel for liquid-liquid extraction cleanup step to remove co-extracts from brinjal extracts.

Preparation of the spiking solution

In a pre-weighed scintillation vial, solvent acetone was taken and dissolved ^{14}C-chlorpyriphos (with specific activity = 26.8 mCi/ mmol) having radioactivity equal to 1 µCi. To access the amount of radioactivity left in the scintillation vial, the vial used for preparing the spiking solution was also used for radioactivity measurement after the spiking is over

Treating the Brinjal units with ^{14}C-chlorpyriphos

Brinjal units were washed and 1.5 kg weighed units were cut longitudinally into equal halves. These units with their cut surface facing downward were placed on a clean aluminum foil. With the help of Hamilton's microsyringe, the spiking solution so prepared was applied over the peel area of brinjal units and left aside, undisturbed. Surface run-off (solution could not be absorbed on brinjal units) spiking solution from aluminum foil was then collected in a separate scintillation vial for radioactivity measurement, as that too is residue loss.

Processing of Brinjal matrix under different temperature conditions

In the first step of matrix processing, the spiked Brinjal units were chopped continuously for 6-7 minutes in a laboratory chopper by giving 1-minute pauses in-between the runs of the chopper. At this step, the homogeneity of the matrix was ensured by monitoring the peel size of chopped brinjal units and concomitants mixing the processing matrix with a spatula.

Subsequently, in the second step matrix processing, 400g of chopped units and 20 mL of double distilled water were added to a warring blender wherewith its steel blades grinding was carried for 3 minutes. Again the peel size of the brinjal was checked. This was the sample processing for brinjal units under ambient conditions.

During cryogenic processing, that is sample processing under low-temperature conditions instead of adding water, dry ice was added during both the first and second step of matrix processing. Only the required amount of dry ice (measured) was added into the warring blender to obtain a free-flow processed analytical portion of brinjal. The peel size of brinjal was also monitored in both the processing steps during the above-mentioned cryogenic milling.

Sampling out of the analytical portions

For sampling out different analytical portions of processed brinjal matrix, 250 mL thick-walled centrifuge tubes and 1L beakers were taken. The 15g and 150g processed analytical portions were withdrawn from the first step sample processing container that is from the laboratory chopper whereas the 5g and 50g analytical portions were withdrawn from the warring blender that from matrix processed with the second step grinding procedure.

Pesticide residues extraction procedure

For the extraction of the analyte, ^{14}C-chlorpyrifos Sodium bicarbonate was used. The sampled out different analytical portions were added with sodium bicarbonate. The extraction salt was taken in the ratio of 6:1 i.e., for 5g, 15, 50g, and 150g analytical portion, 0.83g, 8.33g, 2.5g, and

25g, sodium bicarbonate was taken. The mixture was then warmed on a hot water bath at 27°C, constantly stirred spatula. Extraction solvent ethyl acetate was added to the salt and matrix mixture in the ratio of 2:1. Sodium sulfate was then added to each analytical portion in the ratio of 1:1 w/w. Each analytical portion was homogenized with a normal laboratory homogenizer, and covered with aluminum foil. The 5g and 15g analytical portions were homogenized for 1-2 minutes and later centrifuged at 2500 rpm for 10 minutes. The 50g and 150g were never homogenized on the machine rather were kept undisturbed. Then 1 mL of supernatant of all the analytical portion extracts containing ^{14}C-Chlorpyrifos was collected in scintillation vials for scintillation counting.

Liquid-liquid partitioning of the brinjal extract as a cleanup step to remove the co-extracts:

This is an additional cleanup step followed to remove the co-extracts like pigments, etc., which may affect scintillation counting efficiency. The solvent used for analyte extraction was non-polar (ethyl acetate) so the liquid-liquid extraction was done by using distilled water (polar). An equal amount to solvent ethyl acetate and distilled water was taken in a separating funnel and liquid-liquid partitioning was carried out thrice until the pigment (green colored) containing extract becomes a clear solution. Radioactivity count in the distilled water used for liquid-liquid extraction was also done to ensure (if any) loss of pesticide residues in the cleanup step, as such no radioactivity was detected in the said distilled water.

Measurement of radioactivity on liquid scintillation counter:

All the scintillation vials intended to measure the radioactivity were added with 19 mL of scintillation cocktail. These vials were gently vortexed and taken to Beckman's Liquid Scintillation counter for the radioactivity measurement. Quench correction was done during scintillation counting to overcome the effect of co-extracts (Pujol and Sanchez-Cabeza, 1997).

Results and Discussion

The quadratic factors introduced by Maestroni et al. in 2018 include conditions applied during the conduct of experiments such as the speed of centrifugation, extent of shaking & agitation, etc. all were taken into consideration. In the present case of study food matrix brinjal processed in two different temperature conditions i.e., ambient and cryogenic showed high percentage recoveries of analyte pesticide and reproducibilities. For the solid-phase extraction in ethyl acetate solvent, the salts sodium hydrogen carbonate and sodium sulfate were used (Viana, 1996).

Special statistical software was procured for complex calculations based on well-defined algorithms (Meier, & Zund, 1993 and Miller, & Miller, 2000) suitable to easily the determination of uncertainty and variability about pesticide residue analysis.

K_S or the sampling Constant is concerned with the amount that is withdrawn from a homogenous sample of analyte and the matrix (Ambrus et al., 1996). It refers to the product of the weight of the analytical portion withdrawn from the homogenously processed matrix (W) and square of the coefficient of variance in sample processing i.e., the relative uncertainty of sample processing ($CV_{SP.}$).

The degree or extent of extraction of residual pesticides is governed by the homogeneity of the processed matrix. It is assumed that both, smaller analytical portion and the larger analytical portion were equally homogenized therefore, they are compared.

$$K_{S\,(SM)} = K_{S\,(LG)} \qquad \text{-----------1}$$

$K_{S\,(SM)}$, denotes the sampling constant for small portion size of the processed matrix.

$K_{S\,(LG),}$ denotes the sampling constant for large portion size of the processed matrix.

Since R denotes the average residue concentration (R) of the small and large analytical portion is the same Hence in the expression for K_S, the value CV^2_{SP} can be substituted by S^2.

Deducing the value of $K_{S\,(SM)}$ and $K_{S\,(LG)}$ from above expression,

For small analytical portion, $K_{S\,(SM)} = W_{SM} \times S^2_{SM}$

For larger analytical portion, $K_{S\,(LG)} = W_{LG} \times S^2_{LG}$

It implies that:

$$S^2_{LG} \times W_{LG} = S^2_{SM} \times W_{SM}$$

Therefore,

$$S^2_{LG} = S^2_{SM} \times \frac{W_{LG}}{W_{SM}}$$

$$V_{SP\,(LG)} = V_{SP\,(SM)} \times \frac{W_{SM}}{W_{LG}} \qquad \text{---------------------- 2}$$

For checking the homogeneity of chpping F-test was applied to compare the variance of sample processing of large versus small analytical portions.

The average recovery (denoted as R) of each analytical portion was analyzed after extraction and then calculated the value of V_A (denotes the average of variance of each sub-sample taken in the vial) and V_T (denotes the average variance of each sub-sample of each analytical portion).

Further, one tail F-test$_{0.005}$ was applied to

$$F_{Calc.} = \frac{V_T}{V_A}$$

When $F_{Calc.} > F_{Tab.}$

It implies that, $V_T >> V_A$

Then V_{SP} (Variance of sample processing) = $V_T - V_A$

And CV_A (Coefficient of Variance of sample processing) = $\sqrt{(V_{SP})}/R$

Where CV_A % signifies the reproducibility percent of the sample processing.

To verify the homogeneity of both the chopper and warring blender, two tail $F_{0.1}$ tests were applied.

$$\text{F-test} = \frac{W_{LG}}{W_{SM}} \text{ X } \frac{W_{SP\,LG}}{W_{SP\,SM}} \qquad \text{(From Equation 2)}$$

$F_{Tab.(0.1,4,4)}=6.39$

When $F_{Calc.} < F_{Tab.}$, it implies that the sample is well mixed, then sampling constant for each process i.e. K_S1 and K_S2 were calculated. Values of CV_1^2 and CV_2^2 can be calculatedwith the help of the computed values of K_S1 and K_S2 respectively, by using the following formula:

$$\mathrm{CV}_1{}^2 = \frac{K_S 1}{W_{WB}}$$

$$\mathrm{CV}_2{}^2 = \frac{K_S 2}{W_A}$$

Where $\mathrm{CV}_1{}^2$ denotes, Coefficient of Variance for chopping.
$\mathrm{CV}_2{}^2$ denotes, Coefficient of Variance for blending.
W_{WB} denotes, Weight of the chopped sample transferred to the warring blender.
W_A denotes, Weight of the analytical portion taken for the analysis.
By summing up the Combined Uncertainty during sample processing:

$$\mathrm{CV}_{\mathrm{Comb.\ sp}} = \sqrt{\frac{K_S 1}{W_{WB}} + \frac{K_S 2}{W_A}} \ \ \mathrm{OR} \ \ \sqrt{(CV_1)^2 + (CV_2)^2}$$

Recovery studies of ^{14}C labeled Chlorpyriphos:

Figure 1 graph represents the trends of percentage recovery for the brinjal matrix processed at two temperature conditions. The figure and the table show the percentage recovery of each analytical portion, both in ambient and cryogenic processing. Analytical portions 15g and 150g showed high recovery percentages, i.e., 52.11% and 51.78% in ambient and 44.46% and 52.06% in dry ice conditions. On the other hand, analytical portions 5g and 50g showed recovery of 36.37% in ambient and 31.26% with dry ice. The 5g and 50g analytical portions recovered analyte pesticide, 36.61% under ambient and 31.77% in dry ice condition.

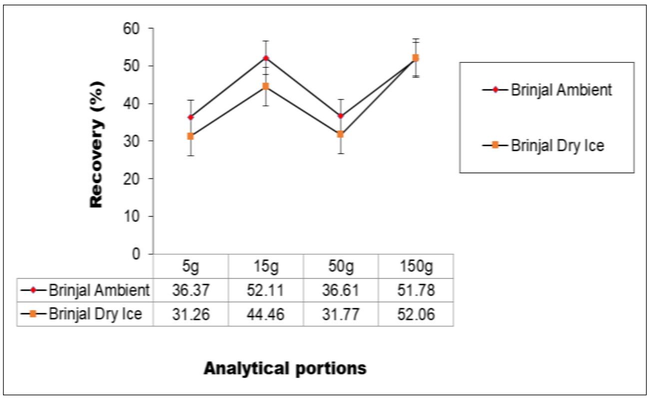

Fig 1: Comparison between Analytical portions and Percentage recovery of ^{14}C- Chlorpyriphos of Brinjal matrix (with respective standard error) processed under two different temperature conditions.

Percentage Reproducibility

As shown in Figure 2, the reproducibility varied differentially in different analytical portions of the brinjal matrix processed under diverse temperature conditions. The highest reproducibility percentage, 7.8, was obtained from 150g, and the least one is 3.3 from 50g analytical portion processed under ambient environment. The trends found were quite staggered. Further, in 15g analytical portions processed with dry ice, the reproducibility was 6.4%, which is better than the other analytical portions where the sample was either processed in ambient or cryogenic temperature.

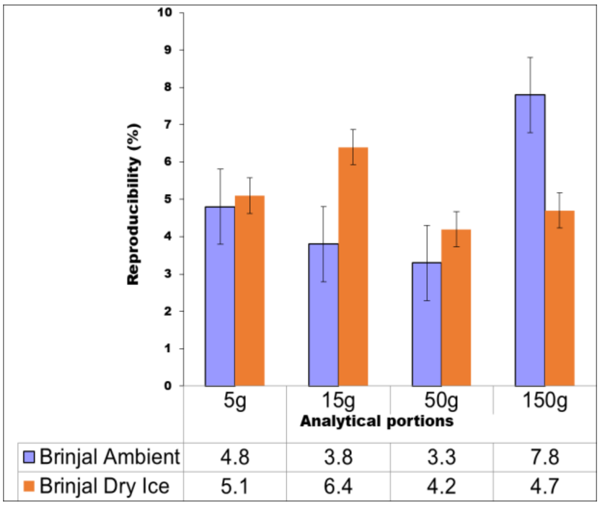

Fig 2: Comparison between Analytical portions and Percentage reproducibility of ^{14}C- Chlorpyriphos of Brinjal matrix (with respective standard error) processed under two different temperature conditions.

Homogeneity in sample processing and determination of Combined Uncertainty

The values of K_S (Sampling constant) and combined uncertainty ($\mathrm{CV}_{\mathrm{COMB.\ SP}}$%) are related to each other as homogeneity of the analyte in the matrix, and uncertainty or variability arising due to various error forms mentioned earlier affects both these parameters equally and simultaneously. The graph shown in Figures 3 and 4 signifies that the homogeneity of chlorpyrifos with brinjal matrix under processing remained the same in both the phases of sample processing. The step involving chopping and grinding in the warring blender did not show variations in their sampling constants or their coefficient of variance during sample processing. Therefore the values of K_S1 and K_S2 obtained were almost the same. It implies that similar values for $\mathrm{CV}_{\mathrm{SP}}$ % are also the same in both the set of temperature conditions selected for sample processing. These findings were unique as generally while working with biological systems, such coinciding values obtained seldom.

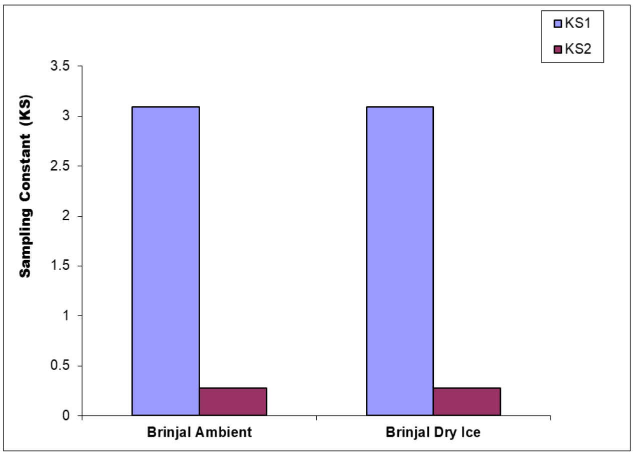

Fig 3: Comparison of sampling constant of Brinjal at different temperature conditions, obtained after two step sample processing.

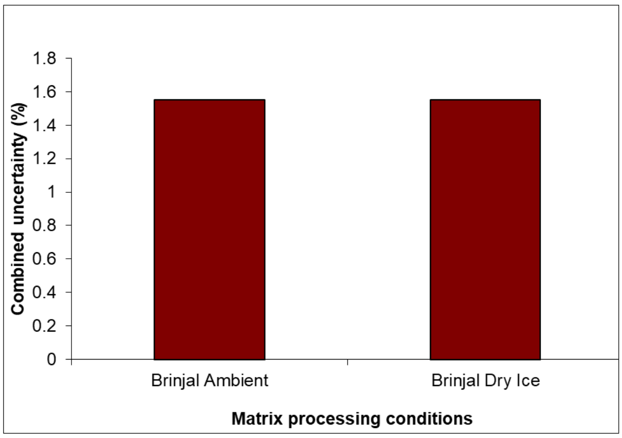

Fig 4: Comparison of combined uncertainty with Brinjal during their sample processing conditions, obtained after two step sample processing.

Conclusion

The maintenance of reproducibility in pesticide residue analysis is indispensable to monitor the repeatability of an experiment and precision in the results. As they account for the origin of uncertainty, both these parameters are tested stringently for residue levels variability in their analysis. Therefore, an approach that consistently yields reproducible results is mandatory for the standardization of protocols. The use of radiolabeled analyte brings sensitivity to analytical methods; hence is crucial for method validation. An ideal method recommended for pesticide residue analysis should characteristically have the least number of steps, take care while handling large sample size, reduce loss & degradation of analyte during sample processing, and be minimally affected due to environmental conditions. A state-of-the-art technique that could unambiguously provide solutions to the problems arising in pesticide residues studies is required.

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References

1. Ambrus Á, Suszter G. Quality control and Quality assurance. In Analysis of pesticides in food and environmental samples, 2019. 2nd edition: CRC Press
2. Ambrus A, Kerekes K, Szemánné-Dobrik H, Domák Z. Sources of random variation of pesticide residue analytical results. Journal of AOAC international,2020:104(2):526-538.
3. Ambrus Á, Solymosne EM, Korsos I. Estimation of uncertainty of sample preparation for the analysis of pesticide residues. Journal of Environment Science Health,1996:31:443-450.
4. Aysal P, Ambrus A, Lehotay SJ, Cannavan A. Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. Journal Environmental Science and Health Part B,2007:42:481-490.
5. Barwick VJ, Ellison SLR. Measurement uncertainty: Approaches to the evaluation of uncertainties associated with recovery. The Analyst,1999:124:981-990.
6. Bidaoui-El M, Jarju OP, Maestroni B, Phakaeiw Y, Ambrus Á. Testing the effect of sample processing and storage on the stability of residues. In Principles of Method Validation; Fajgelj, A.; Ambrus, A., Eds.; Royal Society of Chemistry: Cambridge, UK, 2000, 120-127.
7. Caron F, Benz ML. Analysis and processing of samples for a carbon-14 monitoring program at a radioactive waste storage site. The Analyst,2002:127:1121-1128.
8. Caudros-Rodr'ýguez L, Torres MEH, Lopez EA, Gonzales FJE, Leibanas Vidal JLM. Assessment of uncertainty in pesticide multi-residue analytical methods: Main sources and estimation. Analytica Chimica Acta:2002:454:297-314.
9. da silva RJNB, Lino MJ, Ribeiro A, Santos JR, Filomena M, Camoes GFC. Estimation of precision and efficiency mass transfer steps for the determination of pesticides in vegetables aiming at the expression of results with reliable uncertainty. The Analyst,2000:125:1459-1464.
10. Dornseiffen JW, Van Eck WH. Pesticide residue variability- implications for codex MRLs and world trade. Food additive and contamination,2000:17(7):649-652.
11. Egea González FJ, Hernández Torres ME. Cuadros Rodríguez L, Almansa López E., Martínez Vidal J.L., Estimation and correction of matrix effects in gas chromatographic pesticide multiresidue analytical methods with a nitrogen-phosphorus detector, The Analyst,2002:127:1038-1044. https://doi.org/10.1039/b201461k.
12. Ellison SLR, Gregory S, Hardcastle WA. Quantifying uncertainty in qualitative analysis. The Analyst,1998:123:1155-1161.
13. Ellison SLR, Barwick VJ. Using validation data for ISO measurement uncertainty estimation, Part 1. Principle of an approach using cause and effect analysis. The Analyst,1998:123:1387-1392.
14. Ellison S.L.R. and, Williams A. Quantifying uncertainty in analytical measurement. In EURACHEM/CITAC Guide,2012:third edition:4-6
15. Erney DR, Gillespie AM, Gilvydis DM, Pool CF. Explanation of the matrix-induced chromatographic response enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot on-column injection and flame photometric detection, Journal of Chromatography A,1993:638:57–63.
16. EU Reference Laboratories for Residues of Pesticides. Method validation and quality control procedures for pesticide residues analysis in food and feed, 2006:Quality control procedures for pesticide residues analysis.
17. EURACHEM/CITAC. Quantifying uncertainty in analytical measurements, 2nd edn, 2000. Available online at http://www.measurementuncertainty.org
18. Fussell R, JacKson-Addie K, Reynolds SL, Wilson MF. Assessment of the stability of pesticide during cryogenic sample processing. 1. Apple. Journal of Agriculture and Food Chemistry,2002:50(3):441-8.

19. Hajsˇlova´ J, Holadova´ K, Kocourek V, Poustka J, Godula M, Cuhra P, *et al*. Matrix-induced effects: a critical point in the gas chromatographic analysis of pesticide residues. Journal of Chromatography A,1998:800:283-295
20. Harris CA, Mascall JR, Warren SFP, Crossley SJ. Summary report of the international conference on pesticide residue variability and acute dietary risk assessment. Food additives and contaminants,2000:17(7):481-485.
21. Herrman JL. Acute risk assessment: evolution within the Codex committee on pesticide residues. Food additive and contamination,2000:17(7):551-555.
22. Hill ARC. Residue variability and sampling- practical problems and consequences for residue monitoring. Food additive and contamination,2000:17(7):539-546.
23. Holland PT, Boyd AJ, Malcolm CP. Principles and practices of method validation. The royal society of Chemistry. Cambridge. U.K, 2000, 29-40
24. Hoogerbugge R, Zoonen PV. Principles and practices of method validation. The royal society of Chemistry. Cambridge. U.K, 2000, 19-28.
25. Hortwiz W. Principles and practices of method validation. The royal society of Chemistry. Cambridge. U.K, 2000, 1-8.
26. Hortwiz W. Practicability in regulatory analytical chemistry. Analytical chemistry,1979:51(7):741A.
27. Hortwiz W. Sampling and preparation of sample for chemical examination. Journal of Association official Analytical Chemistry,1988:71(2):241-245.
28. Lantos J, Ambrus A, Visi E, Main factors influencing reproducibility of Multi-residue methods, 1983, 129-134.
29. Maestroni B, Vazquez AR, Avossa V, Goos P, Cesio V, Heinzen H, Riener J, *et al*. Ruggedness testing of an analytical method for pesticide residues in Brinjal. Accreditation and quality assurance,2018:23(5):303-316.
30. Maestroni B, Ghods A, El-Bidaoui M, Rathor N, Ton T, Ambrus A. Testing the efficiency and uncertainty of sample processing using 14C labeled chlorpyrifos, Part II. In Principles of Method Validation; Fajgelj, A.; Ambrus, A. Eds.; Royal Society of Chemistry: Cambridge, UK, 2000, 59-74.
31. Meier PC, Zünd RE. Statistical Methods in Analytical Chemistry, John Wiley & Sons, Inc, 1993.
32. Miller JN, Mille JC. Statistics and Chemometrics for Analytical Chemistry, 4th ed, Pearson Education Limited, 2000.
33. Pujol L, Sanchez-Cabeza JA. Role of Quenching on Alpha/Beta Separation in Liquid Scintillation Counting for several high capacity cocktails. The Analyst,1997:122:383-385.
34. Schenck FJ, Lehotay SJ, Does further clean-up reduce the matrix enhancement effect in gas chromatographic analysis of pesticide residues in food?, J. Chromatogr. A,2000:868:51-61. https://doi.org/10.1016/S0021-9673(99)01137-1.
35. Stepan R, Hajslova J, Kocourek V, Ticha J. Uncertainties of gas chromatographic measurement of troublesome pesticide residues in apples employing conventional and mass spectrometric detectors. Analytica Chimica Acta,2004:520(1-2):245-255.
36. Suhre FB. Variability in pesticide residues- the US experience. Food additives and contaminants,2000:17(7):497-501.
37. Suman P. and Singh D. K., Estimating the uncertainty of pesticide residue analysis from mango using multi-residue analysis and validation of method, Toxicological and Environmental Chemistry,2000:93:1880-1896. https://doi.org/10.1080/02772248.2011.606971.
38. Suszter G, Ambrus A, Schweikert MT, Kalus P.M. Estimation of efficiency of processing soil samples for pesticide residues analysis. Journal of Environmetal Science and Health B,2006:41(5):531–552.
39. Thrope SA, Reynolds SL. Accreditation in the UK for pesticide residue analysis in foods. Journal of Chromatography A,1996:737:3-7.
40. Tiryaki O, Baysoyu D. Estimation of efficiencies and uncertainties of the extraction and cleanup steps of pesticide residue determination in cucumber using 14C-Carbaryl. Accreditation and Quality Assurance,2008:13:91-99.
41. Tiryaki O, Baysoyu D, Seçer E, Aydın G. Testing the stability of pesticides during sample processing for the chlorpyrifos and malathion residue analysis in cucumber including matrix effects. Bulletin of Environmental Contamination and Toxicology,2008:80(1):38-43.
42. Tiryaki O, Seçer E, Aydın G, Baysoyu D. Uncertainty measurement of sample processing of tomato by using 14C-chlorpyrifos for pesticide residue analysis. Fresenius Environmental Bulletın,2009:18(5a):770-775.
43. Viana E, Molto JC, Font G. Optimization of matrix solid-phase dispersion method for the analysis of pesticide residues in vegetables. Journal of Chromatography A,1996:754:437-444.
44. Visi E. Quality assurance/quality control in pesticide residue laboratories. In: Lectures/Possibilities of controlling the various analytical steps. 2002: Training Workshop on Introduction to QC/QA measures in Pesticide Analytical Laboratories, Seibersdorf, Vienna, 2002.
45. Wan HB, Wong MK. Minimization of solvent consumption in pesticide residue analysis. Journal of Chromatography A,1996:754:43-47.
46. Youden W. The role of statistics in regulatory work. Journal of the Association Official Analytical Chemists,1967:50:1007-1013