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UNCERTAINTY ASSOCIATED TO THE ANALYSIS OF PESTICIDE RESIDUES USING SEVERAL APPROACHES: A CASE STUDY

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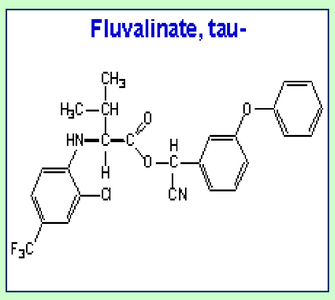
Introduction

Measurement uncertainty is a quantitative indicator of the confidence in the analytical data and describes the range around a reported or experimental result within which the true value can be expected to lie within a defined probability.

Several approaches can be used to estimate the measurement uncertainty associated to the analysis of pesticide residues: a) *top-down* approach where the estimation can be based on default values, the main ways include the Horwitz equation or fit-for-purpose relative standard deviation (FFP-RSD); b) *bottom - up* approach where the estimation is function of the uncertainty sources.

As regards *bottom - up* approach, we have investigated the following contributions: weight of sample, calibration solutions, final volume of sample and intermediate repeatability studies.

The commodity/residue combination selected in this study was celery / tau-fluvalinate pesticide.



Tau-fluvalinate is a broad-spectrum insecticide in the pyrethroid class of pesticides. The Maximum Residue Limit (MRL) of tau-fluvalinate in celery has been set at 0.01 mg/kg (Reg. n. 39672005 of the European Parliament and Commission Reg. n. 149/2008) .

The presented work compares the uncertainty estimated by experimental data using repeated analysis (n = 12) of a real sample and a spiked sample. We have analysed samples of celery containing residues of the pesticide tau-fluvalinate at about 0.1 – 0.5 mg/kg; another sample of celery found free (at 0,01 mg/kg) from residues of the investigated pesticide was fortified at a concentration level (0.1 mg/kg) near the value found in the incurred samples and analysed in 12 replicates.

The quantification of tau-fluvalinate residues in celery was performed by QuEChERS method (acetonitrile extraction/partitioning and dispersive SPE cleanup) followed by GC-MS/MS (QQQ) determination.

Experimental

Analytical Procedure

Extraction

- ✓Weight 10 g of portion homogenized celery into a 50 mL centrifugation tube.
- ✓ Add 10 mL of acetonitrile and mix by vortex.
- ✓Add 4 g $MgSO_4$, 1 g NaCl, 1 g Sodium citrate tribasic dihydrate and 0.5 g Sodium citrate dibasic sesquihydrate.
- ✓Centrifugate (10 min; 5000 rpm)

Purification SPE – Dispersive

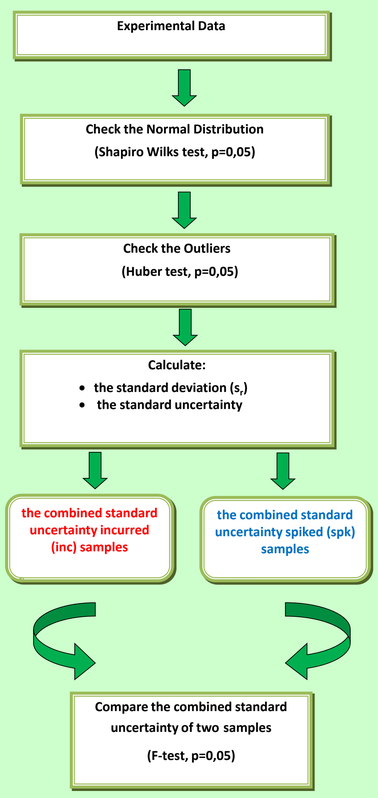
- ✓Transfer an aliquot of 6 mL of the acetonitrile phase into a 15mL centrifugation tube already containing 150 mg PSA and 900 mg $MgSO_4$.
- ✓Mix by vortex
- ✓Centrifugate (2 min; 6000 rpm)

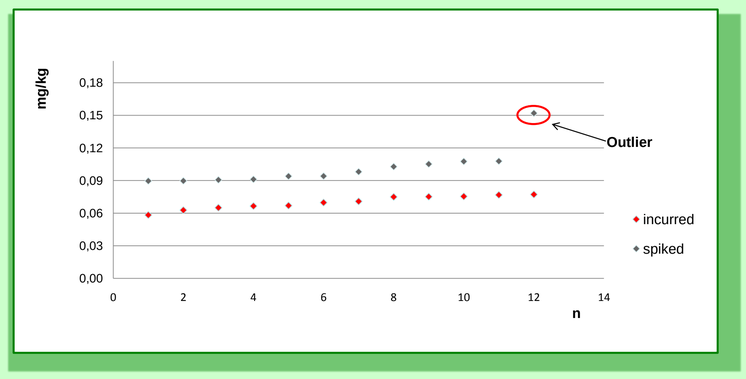
Determination

- ✓ GC/MS-MS(QQQ) triple quadrupole Model 7000 Agilent Technologies
- ✓ PTV injection
- ✓ Capillary column DB-5 (30m x 0.25mm x 0.25μm)
- ✓ External matrix - matched calibration
- ✓ Single – level calibration

Instrumental parameters	
Retention Time (min)	
Peak 1	34.83
Peak 2	34.97
Response Factor (n=8; mean ± s.d)	
Peak 1	51700 ± 7600
Peak 2	45400 ± 6800
MRM transitions (m/z)	250.0 / 55.0 250.0 / 200.0 208.9 / 141.1
Quantifying ions (m/z)	208.9 → 141.1

Statistical Analysis





Results of Statistical Analysis	Incurred Samples	Spiked Samples
n	12	11
Mean (X_m, mg/kg)	0.070	0.097
Standard deviation (s_r)	0.0062	0.0073
Variance (s_r^2)	3.74E-05	5.299E-05
Relative standard deviation (CV %)	8.9	7.5
Rec (%)	-	97
Minimum (Min, mg/kg)	0.058	0.090
Maximum (Max, mg/kg)	0.077	0.108
Median (mg/kg)	0.070	0.094
Freedom degree (ν= n-1)	11	10

Results and Discussion

The tau- Fluvalinate showed two chromatographic peaks.

The individual standards are not available, consequently the instrumental responses of two peaks are summed. The total residue is calculated on the basis of summed peaks.

This approach assumed that all components included in the residue definitions have the same response factors (calculated as height/concentraction) of the detection system. Comparing the variances between the response factors of two peaks by an F-test at the 95% significance level did not reveal any differences (F obs = 1,27 < F crit $_{(\nu1=7;\ \nu2=7;\ p=0,05)}$= 2,85).

Some statistical tests were performed in order to estimate the uncertainty.

The normality of the data was checked by the Shapiro - Wilks test (significance level α=0,05) and the identification of the possible outliers by the Huber test (significance level α=0,05).

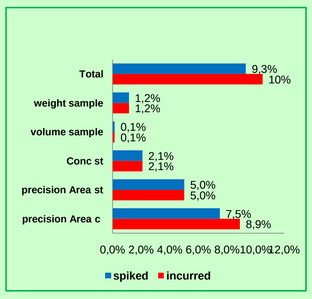
The incurred samples showed a normal and homogeneous distribution, while only an anomalous data was identified in the spiked samples.

No significant differences in the combined standard uncertainty were observed from the two data set (incurred and spiked), in fact the observed value of F has proved to be less than the critical value (F obs = 1,52 < F crit $_{(\nu1=16;\ \nu2=15;\ p=0,05)}$= 2,35).

Any differences were observed in the dispersion of repeated determinations of real samples and simulated experimental samples.

The relative expanded uncertainty for two data set, incurred and spiked, was 22% and 20%, respectively.

The precision of $Area_c$ expressed as relative standard deviation was the component that has contributed with the highest percentage value respect to the other sources of uncertainty.



Uncertainty estimations	Incurred	Spiked
Conc mg/kg	0.070	0.097
Relative standard uncertainty A_c	8.9%	7.5%
freedom of degree	11	10
Relative standard uncertainty Ast	5.0%	5.0%
freedom of degree	4	4
Relative standard uncertainty C_{st}	2.1%	2.1%
freedom of degree	inf.	inf.
Relative standard uncertainty V	0.1%	0.1%
freedom of degree	inf.	inf.
Relative standard uncertainty W	1.2%	1.2%
freedom of degree	inf.	inf.
Relative combined standard uncertainty (u_c)	10%	9.3%
Combined standard uncertainty (uc) mg/kg	0.0073	0.0091
effective freedom of degree	16	15
Coverage Factor (k)	2.12	2.13
Expanded uncertainty (U) mg/kg	0.016	0.019
Relative expanded uncertainty (U)	22%	20%

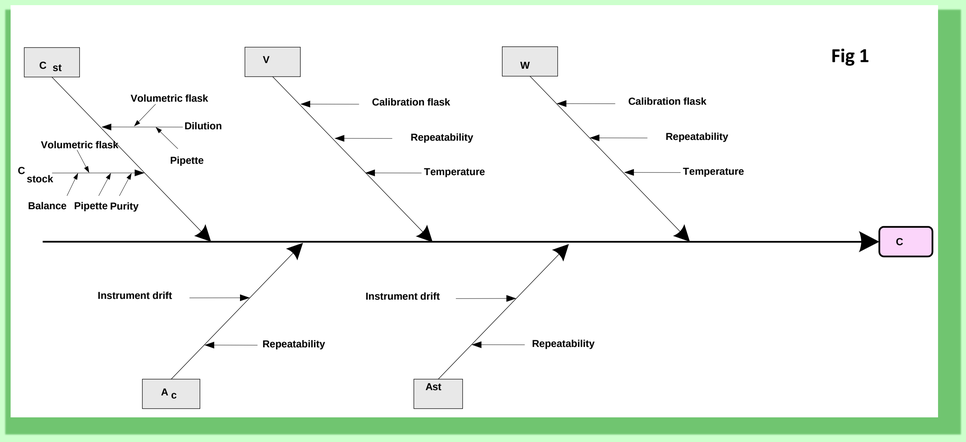


Fig 1

The sources of uncertainty for the method were identified by constructing a cause-and-effect diagram. The "effect" is the result of the analysis, the "cause" is the main parameters controlling the result. The relationship between the result (or the "measurand") and the parameters (or the "input quantities") are shown in Eq. (1) and the cause-and-effect diagram are shown in Fig. (1).

$$C = \frac{A_c}{A_{st}} \cdot C_{st} \cdot \frac{V}{P} \qquad (Eq.1)$$

where

C	**is the concentration of the pesticide in the sample (mg/kg)**
Ac	**is the peak Area of the sample extract**
Ast	**is the peak Area of the reference standard**
Cst	**is the mass concentration of the reference standard (mg/ml)**
V	**is the volume of the sample (ml)**
W	**is the weight of the sample (kg)**

The uncertainties associated with these parameters will contribute to the overall uncertainty in the final result (C) in accordance with law of propagation of uncertainty (Eq. 2):

$$u_{(C)} = \sqrt{\left(\frac{\partial C}{\partial A_c}\right)^2 u_{(A_c)}{}^2 + \left(\frac{\partial C}{\partial A_{st}}\right)^2 u_{(A_{st})}{}^2 + \left(\frac{\partial C}{\partial C_{st}}\right)^2 u_{(C_{st})}{}^2 + \left(\frac{\partial C}{\partial V}\right)^2 u_{(V)}{}^2 + \left(\frac{\partial C}{\partial P}\right)^2 u_{(P)}{}^2} \qquad (Eq.2)$$

Since the Eq. 1 involve only products and quotients, the solution of Eq. 2 is simplified in Eq. 3:

$$\frac{u_{(C)}}{C} = \sqrt{\left(\frac{u_{(A_c)}}{A_c}\right)^2 + \left(\frac{u_{(A_{st})}}{A_{st}}\right)^2 + \left(\frac{u_{(C_{st})}}{C_{st}}\right)^2 + \left(\frac{u_{(V)}}{V}\right)^2 + \left(\frac{u_{(P)}}{P}\right)^2} \qquad (Eq.3)$$