Multi Residue analysis of pesticide residues in Fruits & Vegetables

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Multi Residue Method / Ethyl acetate Extraction

10g sample + 20ml EtOAc + 10g sodium sulfate + 3g $NaHCO_3$

In Falcon polypropylene conical tube

↓

Homogenization with Ultra Turrax for 1 min

↓

Centrifuge at 3300 rpm for 3 min

↓

Freeze at < -15° C for at least 2 h

↓

Sample dilution in MeOH and EtOAc for LC-MS/MS and GC-MS/MS measurement respectively (0.2g sample/ml extract)

Introduction: Measurement Uncertainty (MU) as the qualitative indicator of the confidence in the analytical data, describes the range around an experimental result within which the true value is expected to lie within a defined probability. The expanded measurement uncertainty (MU) was calculated following the approach recommended in Appendix C of the SANTE doc. 11945/2015 using the within laboratory reproducibility data and the results obtained from the EU Proficiency tests.

Objectives:

- ✓ Development and validation of Multi Residue Method for the Pesticide Residues analysis in fruits and Vegetables using LC-MS/MS & GC-MS/MS
- ✓ To provide evidence that the method is fit for the intended purpose

Validation Data & Results

Validation studies have been carried out in both LC-MS/MS & GC-MS/MS analytical systems. For the LC-MS/MS analysis spiked samples of 6 replicates at 2 different levels were analyzed for in total 193 pesticides in 5 different commodities, while in the GC-MS/MS analysis 6 different commodities were studied for 85 pesticides.

The average recoveries of the majority of the compounds were found to be in the range of 70-120% and were characterized by precision lower than 20%.

Estimation of Measurement Uncertainty (MU)

EUPT-FV-18 European Proficiency Test FV-18

EUPT – SRM12 (Matrix: Strawberry Purée)

EUPT-FV-BF01 European Proficiency Test FV-BF01

The expanded MU is calculated using the within-laboratory reproducibility relative standard deviation combined with estimates of the method and the laboratory bias using PT data applying the equation:

$$u' = \sqrt{u'\left(RSD_{WR}\right)^2 + u'\left(bias\right)^2}$$

U′(bias) component calculated from PT results

EUPT	Pesticides	Lab Result mg/kg	Assigned Value mg/kg	(Bias)²	Qn	No of results	√No	Qn/√No
EUPT - FV18, Spinach	Chlorantraniliprole	2,5460	3,071	0,0292	0,18	118	10,8628	0,01657
	Cyazofamid	0,2112	0,279	0,0591	0,22	89	9,434	0,02332
	Difenoconazole	0,6600	0,837	0,0447	0,18	155	12,450	0,01446
	Diflubenzuron	0,9744	1,121	0,0171	0,18	118	10,863	0,01657
	Dimethoate	0,0260	0,036	0,0772	0,17	162	12,728	0,01336
	Omethoate	0,1200	0,142	0,0240	0,25	147	12,124	0,02062
	Famoxadone	0,8408	1,026	0,0326	0,15	122	11,045	0,01358
	Fluopyram	0,2193	0,289	0,0582	0,14	111	10,536	0,01329
	Imidacloprid	0,2450	0,25	0,0004	0,13	146	12,083	0,01076
	Indoxacarb	1,2367	1,436	0,0193	0,19	145	12,042	0,01578
	Metalaxyl	0,0481	0,058	0,0291	0,16	156	12,490	0,01281
	Thiacloprid	0,4657	0,516	0,0095	0,12	144	12,000	0,01000
	Triadimenol	0,1558	0,205	0,0576	0,17	149	12,207	0,01393
EUPT - BF01, Peach Baby Food	Boscalid	0,0130	0,015	0,0178	0,15	48	6,928	0,02165
	Chlorpropham	0,0190	0,02	0,0025	0,17	47	6,856	0,02480
	Chlorpyrifos	0,0130	0,012	0,0069	0,15	51	7,141	0,02100
	Cypermethrin	0,0100	0,01	0,0000	0,28	44	6,633	0,04221
	Cyprodinil	0,0220	0,026	0,0237	0,15	50	7,071	0,02121
	Deltamethrin	0,0340	0,035	0,0008	0,18	52	7,211	0,02496
	Dimethoate	0,0079	0,009	0,0149	0,19	49	7,000	0,02714
	Etofenprox	0,0200	0,024	0,0278	0,17	49	7,000	0,02429
	Fipronil	0,0066	0,01	0,1156	0,22	47	6,856	0,03209
	Heptachlor	0,0064	0,008	0,0400	0,22	47	6,856	0,03209
	HBZ	0,0071	0,009	0,0446	0,18	48	6,928	0,02598
	Imidacloprid	0,0120	0,013	0,0059	0,2	46	6,782	0,02949
	Iprodione	0,0310	0,037	0,0263	0,17	46	6,782	0,02507
	Malathion	0,0250	0,03	0,0278	0,15	48	6,928	0,02165
	Omethoate	0,0130	0,014	0,0051	0,25	48	6,928	0,03608
	Spinosad	0,0110	0,012	0,0069	0,18	47	6,856	0,02626
	Tebuconazole	0,0200	0,025	0,0400	0,12	50	7,071	0,01697
EUPT SRM12, Strawberries	Chlorothalonil	0,1250	0,125	0,0000	0,25	118	10,863	0,02301
	Captan (Sum)	0,3060	0,302	0,0002	0,25	65	8,062	0,03101
	Folpet (Sum)	1,0900	1,195	0,0077	0,21	66	8,124	0,02585
	THPI	0,1070	0,11	0,0007	0,30	68	8,246	0,03638
	Phthalimide	0,5180	0,446	0,0261	0,22	67	8,185	0,02688
	Carbofuran (Sum)	0,0027	0,003	0,0100	0,42	59	7,681	0,05468
		$\sum(\text{bias}_i)^2$		0,9092			$\sum \frac{Qn}{\sqrt{No}}$	0,846
No of results (m) = 36					No of results (m) = 36			
U´(bias) = 0.1616								

Within Laboratory Reproducibility

Is calculated using routine recoveries for 2 methods:

LC-MS/MS with 73 pesticides and GC-MS/MS with 72 pesticides

Average Recovery of all Results	0,885
SD of all Results	0,060
U′(RSD_{WR})	**0,068**

Measurement Uncertainty: U′ = 0,1753

Expanded MU: U′ = k x U′ = 2 x 0,1753 = 0,35

U′ = 35%

Results & Discussion:

- ➢ The validation results of the average recovery and precision are within the acceptable range of the SANTE criteria.
- ➢ The expanded MU of the method was estimated at 35%, lower than the default MU of 50% recommended to be used by the competent authorities in the SANTE guidelines.
- ➢ The method fits for the purpose to be used

Case of practical application of MU in samples exceeding MRL

Case 1: Strawberries

Cypermethrin : found value = 0,55 mg/kg, MRL= 0,07 mg/kg, MU=50%

Result: 0,55 ± 0,28 *The sample exceeds the MRL!*

Case 2: Peaches

Carbentazim: found value = 0,31 mg/kg, MRL = 0,2 mg/kg, MU=50%

Result: 0,31 ± 0,16 *The sample does not exceed the MRL!*