Contribution of sample processing to variability and accuracy of measured residues

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Objectives -highlights

- **Testing the efficiency of sample processing and stability of residues during sample homogenization**
- **Test matrices**: tomato, lettuce and corn, surface treated with test mixture at 0.1 mg/kg and 0.2 mg/kg level
- **Homogenization**: at room temperature (RT) and deep-frozen sample material with dry ice (DI)
- **Test analytes**: mixture of 17-19 pesticide active substances
- **"Stable" reference compounds**: buprofezin, cyprodinil and chlorpyrifos

Basic relationships

Combined uncertainty of the laboratory phase of the determination of pesticide residues

$$CV_{\mathrm{L}} = \sqrt{(CV_{\mathrm{SS}}^2 + CV_{\mathrm{Sp}}^2 + CV_A^2)} \qquad CV_L = \sqrt{CV_{\mathrm{Sp}}^2 + CV_A^2}$$

$$\mathrm{CV_A} = \sqrt{\mathrm{CV_{ex}^2 + CV_{Clu}^2 + CV_{IA}^2}}$$

Uncertainty (not efficiency) of extraction calculated from duplicate analyses of the split extracts (CV'_A) of test portions:

$$\mathrm{CV'_A} = \sqrt{\mathrm{CV_{Clu}^2 + CV_{IA}^2}} \qquad CV'_A = \frac{\left(\frac{\sum \Delta}{\mathrm{n}}\right)}{\mathrm{d_2}} \qquad \Delta = \frac{|C_{i1} - C_{i2}|}{\bar{C}_i} \qquad \mathrm{d_2} = 1.128$$

$$\mathrm{CV_{ex}} = \sqrt{\mathrm{CV_A^2 - CV'^2_A}}$$

Determination of CV_L from the analysis of replicate test portions:

$$CV_L = \frac{S_{\bar{C}_i}}{\bar{C}}$$

The uncertainty of sample processing (variability of residues in test portions):

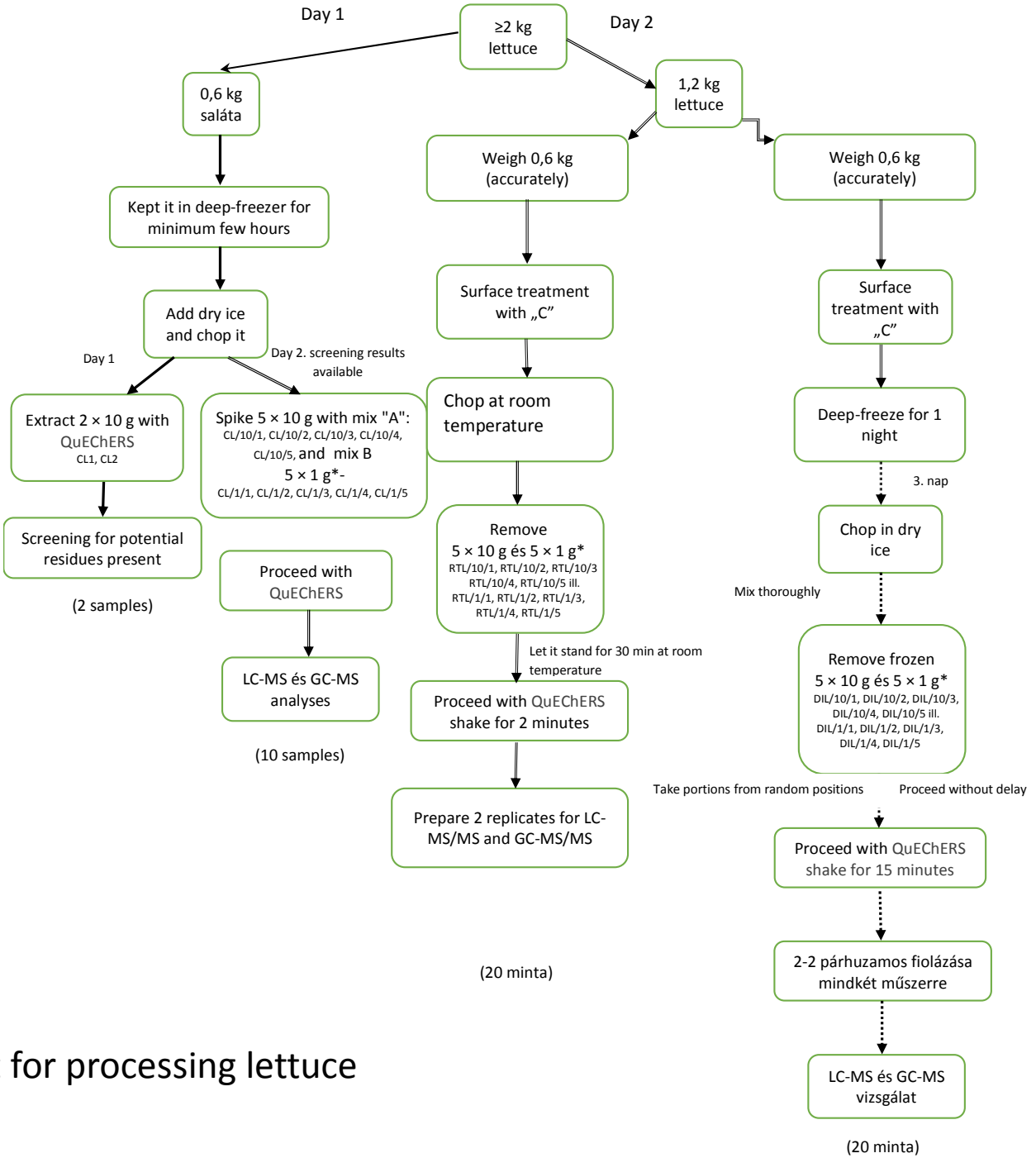
$$CV_{Sp} = \sqrt{CV_L^2 - CV_{rec}^2}$$

Actual concentration of the reference compound $C_{B_1} = \frac{\bar{C}'_{B_1}}{\bar{\bar{C}}_{Brec}}$

The relative concentration of the tested analyte ($C_{i,r}$) during sample processing and analysis is calculated as: $C_{i,r} = \frac{\bar{Q}_r \times C_i}{C_r}$

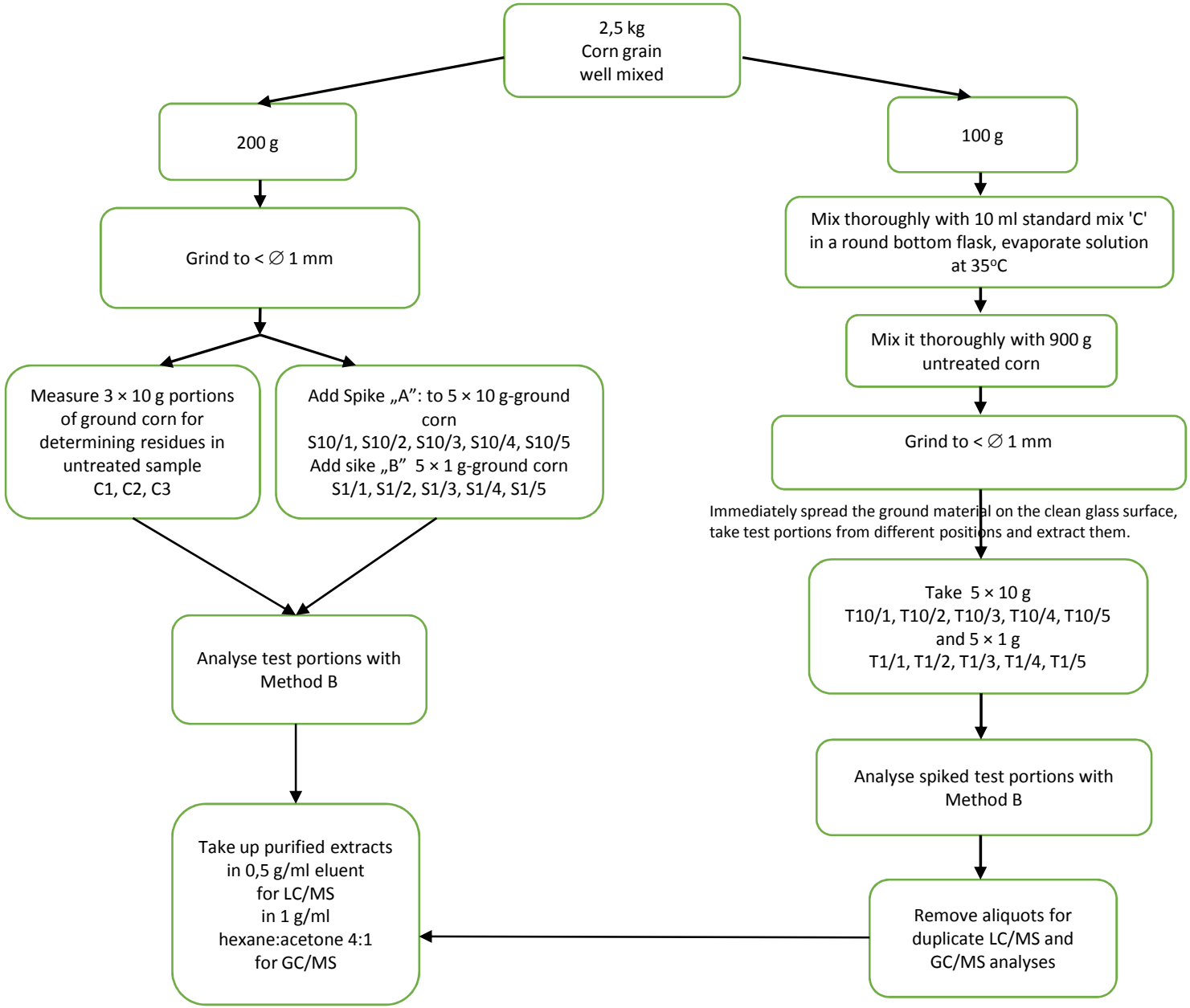
The proportion of j^{th} analytes remained in the processed sample (average of result obtained with 1g and 10 g test portions):

$$C_{j_{rem}} = \frac{\bar{C}_j}{\bar{C}_{ref_j}}$$



Flowchart for processing lettuce

Flowchart for processing corn



RESULTS

Efficiency of chopping

Tomato LC-MS/MS	Average CV_{rec}	Average CV'_{Atestp}	Average CV_L	CV_{Sp}	CV_{Sp1}/CV_{Sp10}
RT 1 g	0.04	0.06	0.08-0.64	0.06-0.63	>4.93
Rt10g	0.05	0.03	0.03-0.08	<0.03	
DI 1 g	0.04	0.04	0.11-0.31	0.10-0.26	2.5
DI10 g	0.05	0.04	0.03-0.12	NS-0.11	
Lettuce GC-MS/MS	**Average CV_{rec}**	**Average CV'_{Atestp}**	**Average CV_L**	**CV_{Sp}**	**CV_{Sp1}/CV_{Sp10}**
RT1 g	0.056	0.068	0.08-0.12	0.150	5.62
RT 10g	0.035	0.043	0.05-0.08	0.027	
DI1 g	0.056	0.079	0.15	0.139	3.66
DI 10 g	0.034	0.030	0.051	0.038	

Efficiency of grinding of corn

0.5-1 mm	68.8 %
0.25-0,5 mm	27.1 %
< 0.25 mm	4.1 %

	Average				
LC-MS/MS	CV_{rec}	$CV'_{A\ testp}$	CV_L	CV_{Sp}	CV_{Sp1}/CV_{Sp10}
1 g	0.055	0.031	0.15	0.136	2.20
10g	0.078	0.029	0.099	0.062	
GC-MS/MS					
1 g	0.042	0.084	0.11	0.10	1.04
10g	0.050	0.074	0.11	0.097	

Performance characteristics of reference compounds

LC-MS/MS		1 g Rec	CV_{rec}	10g Rec	CV_{rec}
Buprofezin	Tomato	0.99	0.031	0.99	0.046
Cyoridinil	Tomato	0.93	0.049	1.01	0.054
Buprofezin	Lettuce	0.88	0.048	1.01	0.045
Buprofezin	Corn	0.93	0.024	0.92	0.083
Cyprodinil	Corn	0.92	0.033	0.88	0.090
GC-MS/MS		1 g Rec	CVrec	10g Rec	CVrec
Buprofezin	Tomato	0.94	0.057	1.06	0.038
Chlorpyrifos	Tomato	0.96	0.049	1.04	0.036
Buprofezin	Lettuce	0.93	0.031	0.94	0.025
Chlorpyrifos	Lettuce	0.78	0.075	0.094	0.025
Cyprodinil	Corn	0.84	0.075	0.75	0.035

Recovery studies were performed at room temperature.

Proportion of analytes remained average of 1 g & 10g

Matrix: tomato, lettuce, corn;

Composition of test mixture:
Buprofezin, Chlorothalonil, Chlorpyrifos, Cyprodinil, Dichlofluanid, Dichlorvos, Etridiazol, Fenhexamid, Heptenophos, Hexaconazole, Chlozolinate, Imidacloprid, Pyrimethanil, Prochloraz, Spiroxamine, Tebuconazole, Tecnazene, Thiacloprid, Trifloxystrobin

Reference compounds: Buprofezin, Cyprodinil

Percentage remained after chopping of tomato:

Tomato	LC-MS/MS		GC-MS/MS	
	RT	DI	RT	DI
Chlorothalonil			nd	nd
Chlorpyrifos			0.86	0.95
Dichlofluanid	nd	nd		
Dichlorvos			0.85	1.02
Etridiazol			0.58	0.86
Fenhexamid	0.55	0.41	0.44	0.49
Chlozolinate			0.32	0.31
Prochloraz	0.81	0.87	0.77	0.81
Tecnazene			0.67	0.72

Percentage reamined after chopping of lettuce:

	LC-MS/MS		GC-MS/MS	
	RT	DI	RT	DI
Dichlorvos			0.77	0.39
Etridiazol			0.67	0.54
Chlozolinate	0.25	0.30	0.94	0.87

Percentage remained after grinding of corn

Corn	LC-MS/MS	GS-MS/MS
Buprofezin	1	1
Chlorpyrifos		0.920
Cyprodinil	0.969	0.969
Dichlorvos	0.712	0.833
Dichlofluanid	NA	
Fenhexamid	0.831	
Heptenophos	0.874	
Spiroxamine	0.761	

CONCLUSIONS

Contribution of extraction step to the combined uncertainty of the results

- There was no significant difference between the repeatability of recovery tests (CV_{rec}) and duplicate analyses of split extracts (CV'_{A}), indicating that the contribution of extraction to the variability of the results is not substantial, and it does not affect the CV_{Sp} estimated from the average residues in test portions and CV_{rec}.

Efficiency of chopping, grinding

- The ratio of the CV_{SP1g}/CV_{SP10g} ranged from 3.7 to 8.5.
- It was influenced by the actual CV_L and CV_{rec} obtained in the tests.
- One set of experiment is not sufficient to obtain typical CV_L values.
- Nevertheless, the results indicate that decreasing the test portion size significantly increase the CV_{SP} and the combined uncertainty (CV_L) of the determination of pesticide residues.
- Reducing test portion below 10-15 g should only be done after verification of the efficiency of sample processing which depends on the equipment used and the matrix analysed, but independent from the analytes.

Efficiency of chopping, grinding (2)

Note that neither the recovery studies nor the proficiency tests results provide information on the CV_L of the laboratory procedure!

The best approach would be, as part of the internal quality control, to regularly reanalyze retained test portions containing detectable residues in various matrices, and calculate the average CV_L based on >15 corresponding tests.

$$CV_{Sp} = \left(\frac{\sum \Delta}{n}\right)/d_2 \qquad \Delta - \frac{|R_1 \quad R_2|}{\bar{R}}$$

D_2=1.128

degree of freedom of estimated standard deviation ($CV_{Sp} \times \bar{R}$) is = 2n

Stability of residues

- Buprofezin, cyprodinil and chlorpyrifos remained >95% in tomato, lettuce and corn.
- Their performance characteristics were stable under the test conditions.
- These substances can be used as reference compounds in further studies.
- The difference between the theoretical concentration (100%) and the proportion remained < 15% could not be statistically confirmed (applying double t-test, and not testing the difference between two mean values).
- Proportion of analytes remained in <85% indicated loss during processing and analysis.

Stability of residues (2)

- The results confirm previous studies that several pesticides undergo degradation during the homogenization process.
- The proportion remaining varied from <LOQ (dichlofluanid) $\rightarrow$ chlozolinate ~ 25-30% $\rightarrow$ to 75-83% (dichlorvos).
- The decomposition/loss of analytes strongly depends on the matrix and temperature of processing.
- Good recoveries do not guaranty stability of analytes, which should be tested for each
analyte - representative matrix - homogenization combinations as part of the validation of the methods.

Closing remarks

- Our limited tests only highlight some of the potential sources which may affect the accuracy and uncertainty of determination of analytes at trace level.
- Several tests performed during the application of a procedure should be performed to obtain more robust information for specific analyte-matrix and test procedure.

Acknowledgement

The tests reported were carried out in two laboratories of the National Food Chain Safety Office. The indispensible contribution of the staff of the laboratories is emphasised and highly appreciated.

Thank you for your attention!
Questions?