CRL for Cereals and Feeding stuff
National Food Institute
Technical University of Denmark

Validation Report 3

Determination of pesticide residues in cereals by GC-MS/MS

(QuEChERS method)

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CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with GC-MS/MS. The method was validated for 24 pesticides, isomers and degradation products in wheat. The method has previously been validated for about 62 pesticides.

The QuEChERS method has an extraction and clean-up step, which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit and vegetables[1].

The method validated here is based on the procedure for dry matrixes (<30% water content) according to the document CEN/TC 275/WG 4 N 0204 (CEN document)(available as a draft). Even though cereals have a fat content of about 2%[2] no attempt has been made to remove the fat from the extract, e.g. freezing out as proposed in the CEN document, since no problems caused by fat has been observed.

2. Principle of analysis

Sample preparation:

Cold water/ice water, acetonitril and an internal standard are added to the milled sample.

Extraction:

The sample is shaken and a salt and buffer mixture is added and the sample is shaken again.

Clean-up:

After centrifugation the supernatant is transferred to a tube with PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract is obtained.

Quantification and qualification:

Internal standard is added and the final extract is analysed GC/MS/MS (electron energy 70 eV, source temp. 180°C, transfer line GC interface 250°C). The injection volume is 8 µl.

Selectivity and specificity:

GC-MS/MS is a highly selective method, and thereby highly specific. Two MRM transitions were used (two parent and two daughter ion) one for quantification and another transition for qualification. Parent and daughter ions are presented in appendix 1.

3. Validation design

The method was validated for 22 pesticides, isomers or degradation products (appendix 1) in wheat.

The validation was performed on 5-6 replicates at each of the three concentration levels. The concentration levels were 0.011, 0.02 and 0.104 mg/kg. A blank sample was included.

4. Chromatograms and calibration curves

Examples of chromatograms obtained when analysing the extracts by GC-MS/MS are presented in figure 1.

The calibration curve is determined by the analysis of each of the 22 analytes at 5 calibration levels, i.e. 0.00289, 0.0087, 0.0289, 0.0868 and 0.289 µg/ml. The calibration curves were best fitted to a linear curve. The majority of the correlation coefficients (R) were higher or equal to 0.98.
Examples of calibration curves are presented in Figure 2.

Five-point matrix-matched calibration curves were used for quantification. The concentration range was between 0.00289 and 0.289 µg/ml. Triphenyl phosphate was used as internal standard and hexachlorbenzen (HCB) was used as quality control standard. Examples of calibration curves are given in Figure 2.

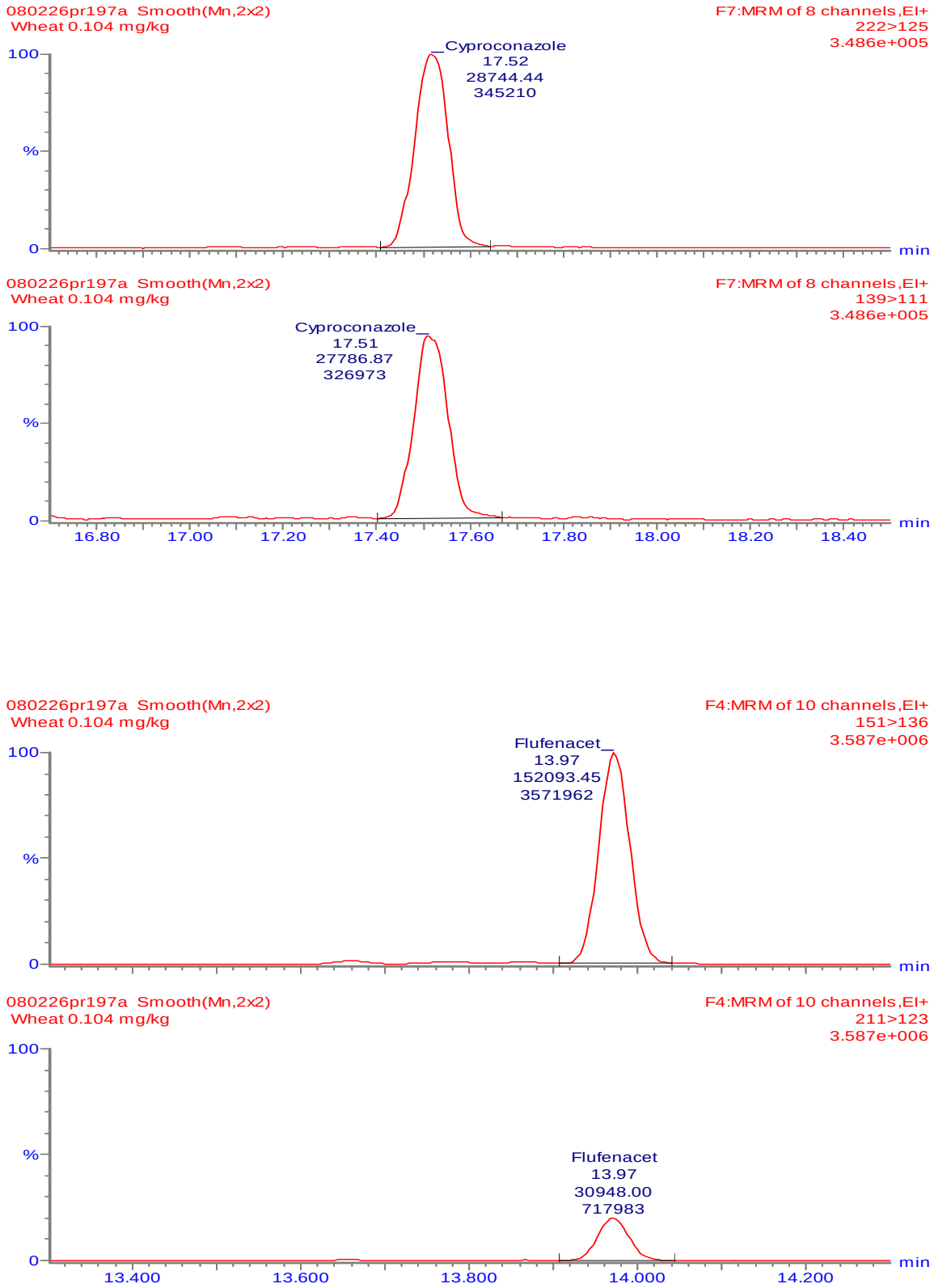

Figure 1: Examples of chromatograms for cyproconazole and flufenacet obtained when analysing extract of wheat spiked with 0.104 mg/kg.

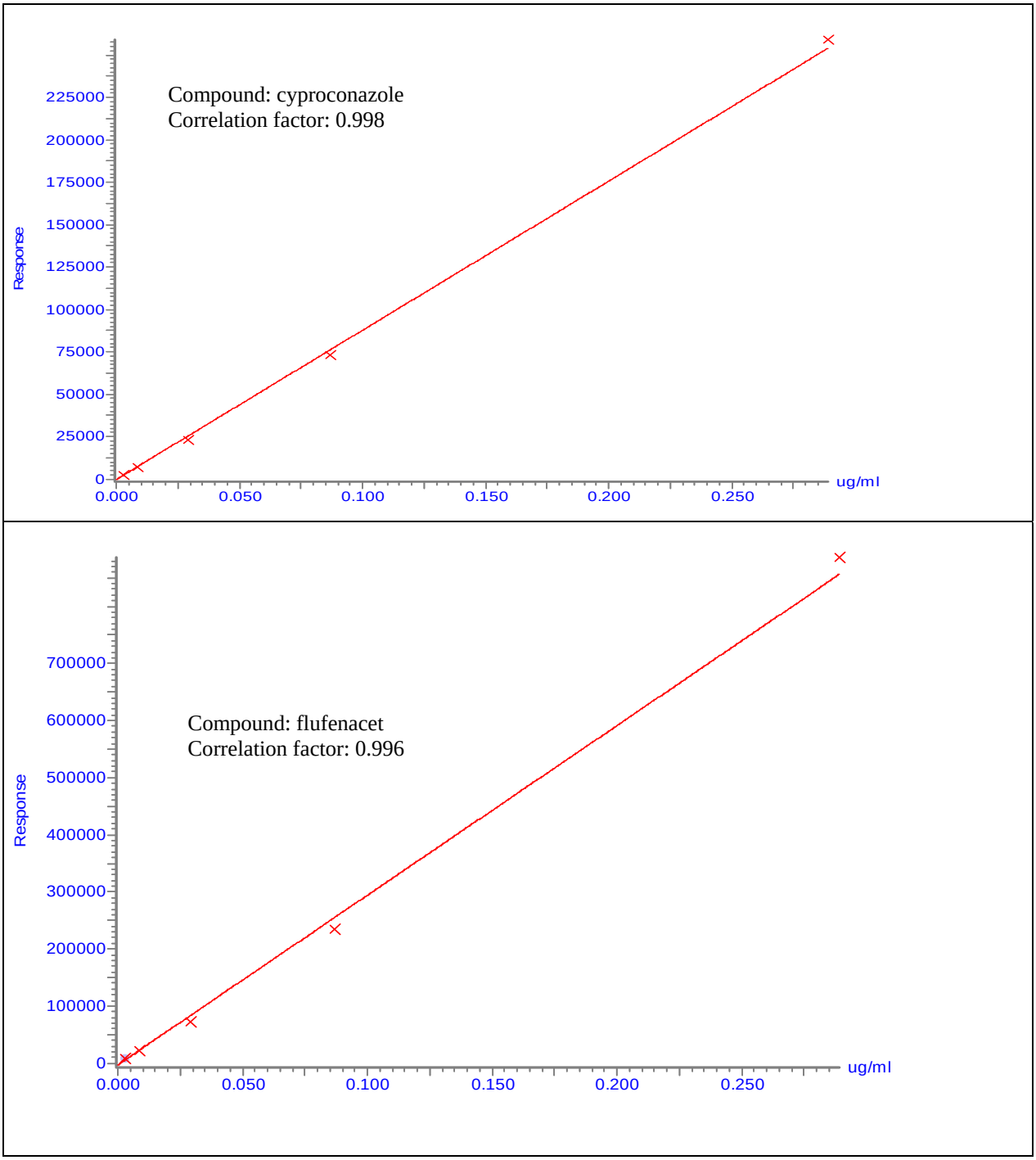

Figure 2. Examples of calibration curves for cyproconazole and flufenacet (concentrations from 0.00289-0.289 µg/ml)

5. Validation parameters

Precision – Repeatability

Repeatability was calculated for all pesticides and degradation products on all three spiking levels.

Repeatability is given as the relative standard deviation on the result from two or more analysis at the same sample, done by the same technician, on the same instrument and within a short period of time. Repeatability in this validation was calculated from the 5-6 replicate determinations.

Repeatability were calculated as given in ISO 5725-2[3].

Appendix 2 shows the relative repeatability for the validated pesticides, isomers and degradation products.

Accuracy – Recovery

The accuracy was determined by recovery, samples were spiked at three concentration levels. In appendix 2 recovery, repeatability and limit of quantification (LOQ) are given for the validated pesticides, isomers and degradation products. For most of the analytes the recovery from wheat were in the range of 75-110% for all three concentration levels (0.011 mg/kg, 0.02 mg/kg and 0.104 mg/kg). Recoveries may be seen in appendix 2.

Robustness

The QuEChERS method has earlier by Anastassiades et al. 20031 in connection with the development of the method been shown to be robust.

Limit of quantification, LOQ

Quantification limits (LOQ) are calculated from the results at the lowest accepted spike level, as 6 times the standard deviation (absolute recovery). The quantification limits are given in appendix 2.

6. Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:

1. The relative standard deviation of the repeatability must be less than or equal to the standard deviation proposed by Horwitz[4].
2. The average relative recovery must be between 70 and 110%[5].

If the above mentioned criteria have been meet, the detection limits have been calculated.

7. Results and discussion

The multi-residue method has been tested for 24 pesticides, isomers and degradation products in wheat.

The relative repeatability (RSD_r) varied between 2 to 30 %, however most of the values were around 7-14%.

For the majority of the pesticides the recovery was in the range of 75-100% at all three concentration levels.

The criteria for acceptance were met for 16 out of 24 pesticides, isomers and degradation products (listed in Table 1). For the triazoles pesticides the LOQs ranged from 0.009 mg/kg to 0.022 mg/kg with a median at 0.013 mg/kg and for the other group of pesticides from the LOQs ranged from 0.010 mg/kg to 0.098 mg/kg with a median at 0.024 mg/kg. The lowest calibration level (LCL) was 0.00289 µg/ml corresponding to LOD at 0.006 mg/kg. However most of the LOQs are above 0.010 mg/kg.

The pesticides, isomers and degradation product which has been validated presented in table 1 are divided in to two groups, one for the compounds for which the acceptance criteria could be meet (Accepted) and those which could not meet the acceptance criteria (Not accepted).

The results for the different pesticides which were accepted are listed in Appendix 1.

Table 1: Compounds validated and not accepted as validated for wheat.

Wheat		
Accepted (14 compounds)		
Cyproconazole	Flusilazole	Trifluralin
Cyprodinil	Flutriafol	Triticonazole
Difenoconazole	Hexaconazole	
Fenbuconazole	Metribuzin	
Flufenacet	Propanil	
Fluquinconazole	Triadimefon	
Not accepted (8 compounds)		
Chlorpropham	Fenpropimorph	Prosulfocarb
Dicofol	Lindane	Tolclofos
Fenpropidin	Metconazole	

The QuEChERS method has in connection with the development been shown to be rugged[1].

8. Conclusions

In conclusion 14 of 22 pesticides, isomers and degradation products were validated for the QuEChERS method using GC-MS/MS for the analysis.

9. References

1 http://www.quechers.com/ or Anastassiades et al., J. AOAC Int., vol. 86, no. 2, p. 412, 2003

2 The Composition of Foods – fourth edition by Erling Saxholt, Gyldendals, 1996.

3 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part 2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

4 W. Horwitz, Anal. Chem., 1982; 54, 67A.

5 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/2007/3131, 31/October/2007, European Commission, Brussels, 2007.

Appendix 1. MRM transitions for the pesticides sought validated.

	Pesticide/metabolite	Parent 1	Fragment 1	Collision energy 1	intensity	Parent 2	Fragment 2	Collision energy 2	intensity
1	chlorpropham	213	171	5	16200000	171	127	5	10500000
2	Cyproconazole	222	125	15	3140000	139	111	15	2030000
3	Cyprodinil	226	225	15	2220000	223	208	15	512000
4	Dicofol	139	111	5	787000	251	139	5	1010000
5	Dicofol-degrad.	139	111	10	20500000	250	139	10	9100000
6	Difenoconazole-1	323	265	15	6520000	325	267	15	3980000
7	Difenoconazole-2	323	265	15	5790000	325	267	15	4020000
8	Fenbuconazole	198	129	10	5260000	129	102	15	2090000
9	Fenpropidin	98	70	10	10700000	99	71	10	442000
10	Fenpropimorph	303	128	5	3160000	117	115	10	739000
11	Flufenacet	151	136	10	15100000	211	123	10	3240000
12	Fluquinconazole	340	298	15	9390000	339	298	15	8860000
13	Flusilazole	314	233	20	2360000	206	137	20	1380000
14	Flutriafol	123	95	10	4500000	219	123	15	3610000
15	Hexaconazole	231	175	10	807000	214	172	15	692000
16	Lindane	217	181	10	5630000	219	183	10	5540000
17	Metconazole	125	89	10	743000	127	89	10	234000
18	Metribuzin	198	82	15	899000	214	198	5	176000
19	Propanil	217	161	5	3080000	161	99	20	2320000
20	Prosulfocarb	251	128	5	5060000	128	86	5	1990000
21	Tolclofos-methyl	265	250	15	14300000	267	252	10	5550000
22	Triadimefon	208	181	5	4190000	181	111	10	624000
23	Trifluralin	264	206	5	8850000	290	248	10	4430000
24	Triticonazole	235	182	15	1010000	217	167	15	529000
IS	TPP	325	169	10	7480000	326	233	10	6820000

Appendix 2. Repeatability, recovery and limit of quantification.

In the tables are presented repeatability and LOQ for the validated compounds. Values outside the acceptance criteria are marked by grey.

Wheat	Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
	0.011	32		0.02	29		0.104	22		
	Recovery, %	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**LOQ**
Trifluralin	146	21		163	12		110	14		0.098
Propanil	96	15		98	8		99	12		0.010
Metribuzin	114	17		123	14		108	9		0.063
Flufenacet	122	15		121	3		112	7		0.047
Triadimefon	120	15		119	4		114	5		0.037
Cyprodinil	113	16		111	8		95	6		0.010
Flutriafol	99	16		89	9		81	7		0.011
Hexaconazole	102	13		95	10		89	7		0.009
Flusilazole	105	13		94	9		86	7		0.009
Cyproconazole	98	16		83	10		76	8		0.010
Triticonazole	91	23		78	9		74	6		0.014
Fluquinconazole	81	25		75	6		75	6		0.013
Fenbuconazole	99	27		89	2		90	5		0.018
Difenoconazole	109	31		98	3		96	6		0.022