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

Validation Report 5

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 22 pesticides, isomers and degradation products in wheat. The method has previously been validated for about 76 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.

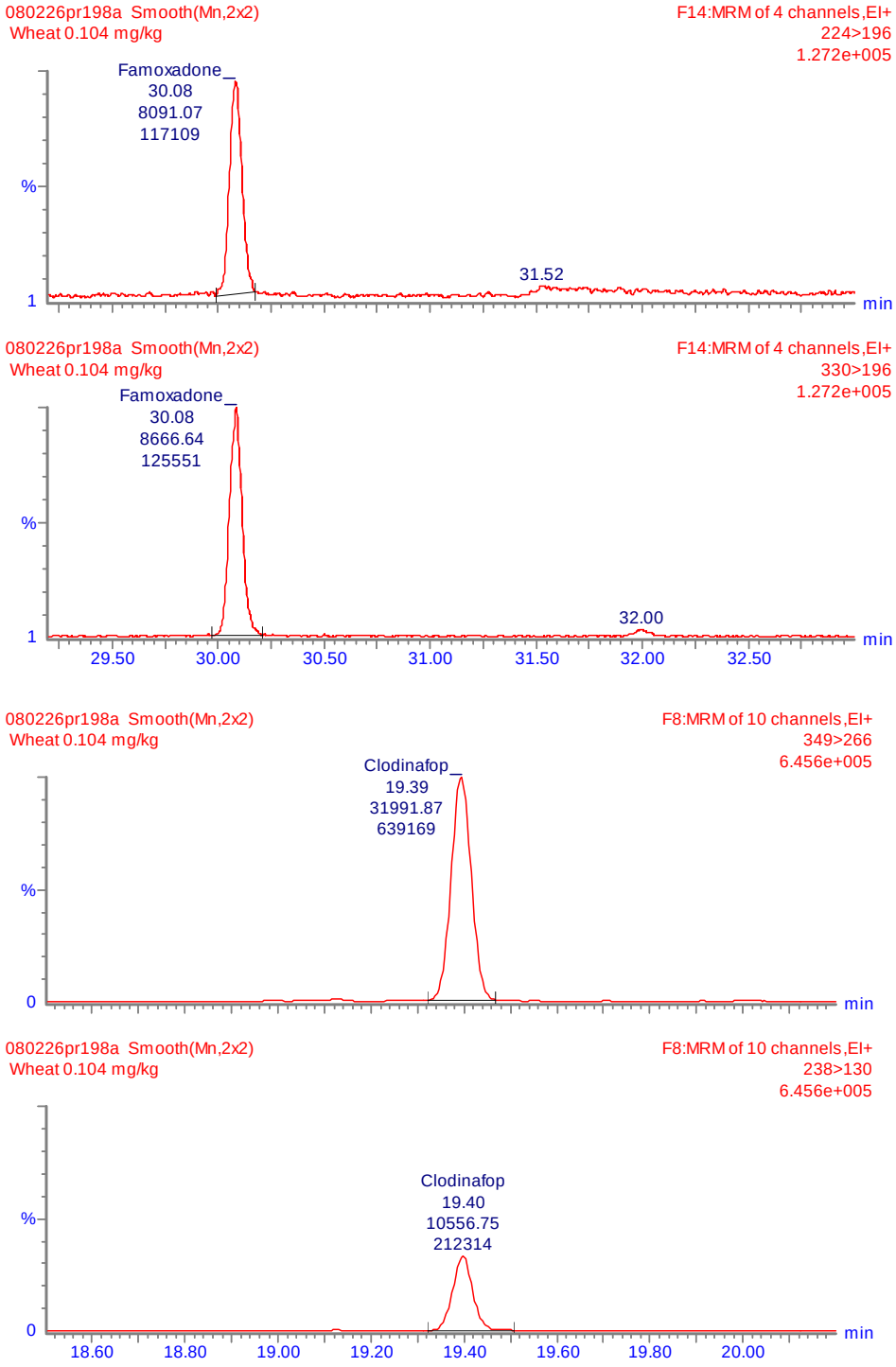

Figure 1: Examples of chromatograms for famoxadon and clodinafop-propargyl obtained when analysing extract of wheat spiked with 0.104 mg/kg.

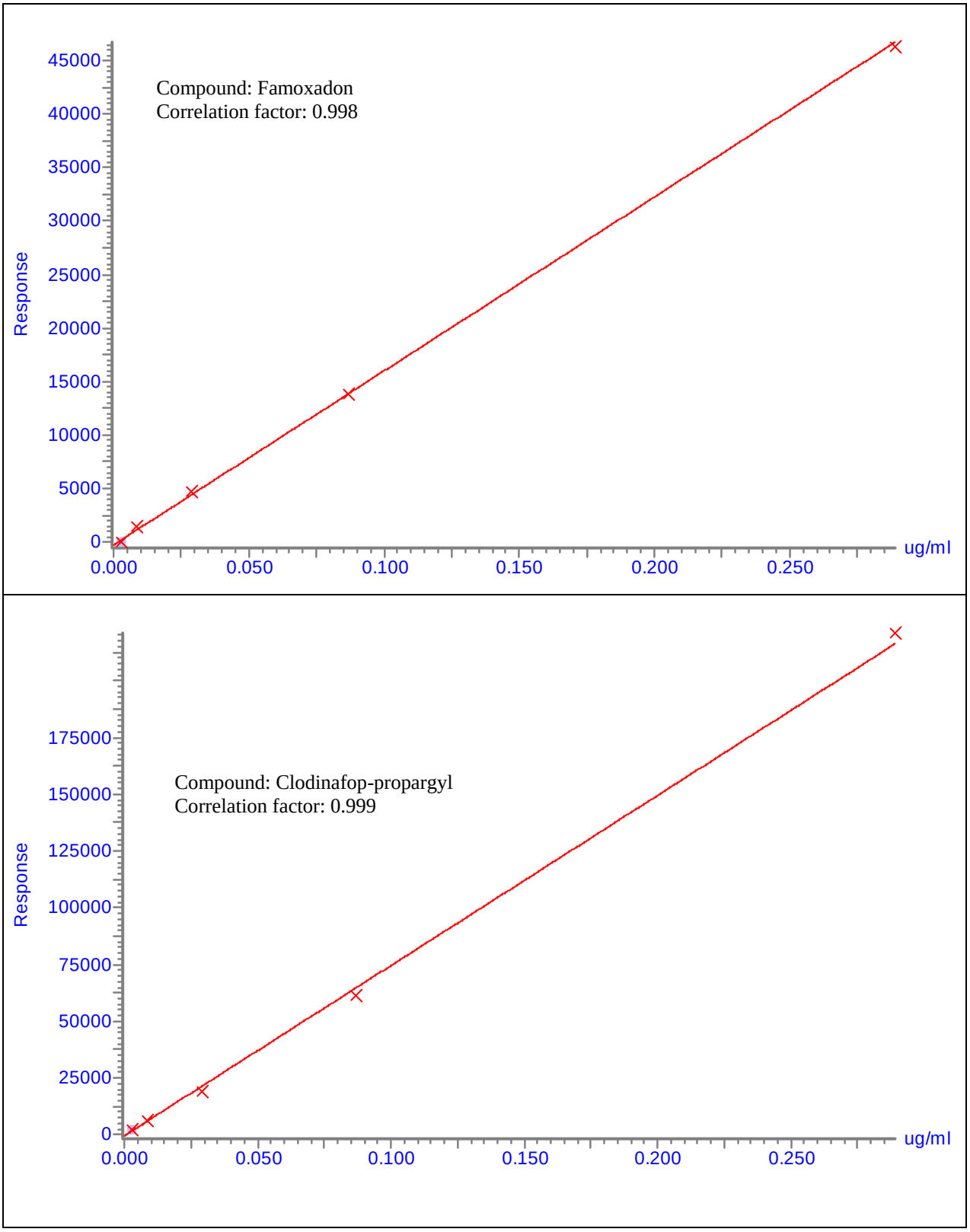

Figure 2. Examples of calibration curves for famoxadon and clodinafop-propargyl (concentrations from 0.00289-0.289 µg/ml)

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 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 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. 2003[1] 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 22 pesticides, isomers and degradation products in wheat.

The relative repeatability (RSD_r) varied between 2 to 21 %, however most of the values were around 5-16%.
For the majority of the pesticides the recovery was in the range of 80-96% at all three concentration levels.

The criteria for acceptance were met for 21 out of 22 pesticides, isomers and degradation products (listed in Table 1). The LOQs ranged from 0.003 mg/kg to 0.015 mg/kg with a median at 0.010 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 at or 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 (21 compounds)		
Fuberidazol	Clodinafop	Fenoxaprop-p
Tetraconazole	Proquinazid	Prochloraz
Triadimenol	Diflufenican	Tau-fluvalinate
Paclobutrazol	Epoxiconazole	Famoxadone
Carboxin	Bromuconazole	Cinidon-ethyl
Flusilazole	Dimoxystrobin	
Diniconazole	Picolinafen	
Quinoxafen	Flurtamone	
Not accepted (1 compounds)		
Fludioxonil		

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

8. Conclusions

In conclusion 21 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	Bromoconazole-1	295	173	10	1260000	293	173	15	1010000
2	Bromoconazole-2	295	173	15	848000	293	173	15	930000
3	Carboxin	235	143	5	10200000	143	87	5	8290000
4	Cinidon ethyl	358	330	10	2100000	330	302	15	1620000
5	Clodinafop-prop.	349	266	10	2250000	238	130	20	603000
6	Diflufenican	394	266	15	14000000	265	246	10	2870000
7	Dimoxystrobin	116	89	15	6960000	205	116	10	6660000
8	Diniconazole	268	232	15	761000	270	232	10	558000
9	Epoxiconazole	192	138	10	3030000	165	138	10	830000
10	Famoxadone	330	196	20	38900000	224	196	10	30400000
11	Fenoxaprop-p	288	119	5	3120000	361	288	10	2820000
12	Fludioxonyl	248	127	20	9000000	154	127	5	3150000
13	Flurtamone	333	120	20	1470000	199	157	15	503000
14	Flusilazole	314	233	20	2360000	206	137	20	1380000
15	Fuberidazole	155	129	20	1220000	184	156	10	6210000
16	Paclobutrazol	236	125	15	1430000	238	127	15	564000
17	Picolinafen	375	238	20	7880000	377	239	15	1370000
18	Prochloraz	180	138	10	418000	308	266	5	224000
19	Proquinazid	288	245	15	16200000	330	288	5	8450000
20	Quinoxyfen	237	208	20	5820000	272	237	15	4940000
21	Tau-fluvalinate-1	208	181	15	3560000	250	200	20	1150000
22	Tau-fluvalinate-2	250	200	20	1040000	208	181	10	368000
23	Tetraconazole	171	136	10	1450000	337	220	15	933000
24	Triadimenol-1	168	70	5	1180000	128	100	10	299000
25	Triadimenol-2	168	70	10	1470000	128	100	10	587000

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 written in italic and by grey background.

Wheat, QuEChERS	Spike level mg/kg 0.011	Horwitz, % 32	Spike level mg/kg 0.02	Horwitz, % 29	Spike level mg/kg 0.104	Horwitz, % 22	
	Recovery, %	**RSDr, %**	**Recovery, %**	**RSDr, %**	**Recovery, %**	**RSDr, %**	**LOQ**
Fuberidazol	91	14	75	6	*65*	*11*	0.008
Tetraconazole	*117*	*18*	113	7	104	5	0.009
Triadimenol	111	15	96	12	93	10	0.011
Paclobutrazol	*120*	*14*	103	7	93	10	0.009
Carboxin	85	15	75	8	*68*	*8*	0.008
Flusilazole	105	13	99	10	86	7	0.009
Diniconazole	104	14	91	12	82	6	0.009
Quinoxafen	93	16	86	12	82	7	0.010
Clodinafop	94	16	87	7	89	9	0.010
Proquinazid	93	16	86	9	95	8	0.010
Diflufenican	92	17	86	8	84	9	0.010
Epoxiconazole	97	16	83	9	74	8	0.010
Bromuconazole	89	17	76	8	72	7	0.010
Dimoxystrobin	96	15	81	11	73	7	0.009
Picolinafen	94	17	84	10	80	6	0.010
Flurtamone	90	19	80	4	82	7	0.011
Fenoxaprop-p	76	19	70	2	80	8	0.010
Prochloraz	107	21	81	7	80	10	0.015
Tau-fluvalinate	93	17	85	9	*68*	*9*	0.011
Famoxadone	*122*	*45*	101	8	101	7	0.010
Cinidon-ethyl	*109*	*36*	92	2	95	5	0.003
Maximum	111	21	113	12	104	10	0.015
Minimum	76	13	70	2	72	5	0.003
Median	93	16	86	8	83	7	0.010
Average	95	16	87	8	86	8	0.010