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

Validation Report 4

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

(QuEChERS method)

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CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with LC/MS/MS. The method was sought validated for 18 pesticides in wheat, and 13 pesticides were accepted in the validation. All the validated pesticides were determined by positive electrospray ionisation (ESI)
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 has already been validated on fruits and vegetables[1], but the data available on cereals is limited.
The present method validated was 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 were added to the milled sample.

Extraction:
The sample was shaken and a salt and buffer mixture was added and the sample was shaken again.

Clean-up:
After centrifugation the supernatant was transferred to a tube with PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract was obtained.

Quantification and qualification:
Internal standard ($^{13}C_6$-carbaryl) was added for quantification. The final extract was analysed LC-MS/MS. The injection volume was 10 µl. Instrument specifications are presented in details in the paper written by Granby et al. 2003[3].

Selectivity and specificity:
LC-MS/MS is a highly selective method, and thereby highly specific. All pesticides were detected in the multiple reaction monitoring mode (MRM). For each pesticide precursor ion and 2 product ions (where possible) were determined. One product ion for quantification and one for qualification. The MRM transitions for the pesticides and degradation products sought validated are given in appendix 1.

3. Validation design

The method was validated on wheat. Recovery tests were all on organic grown wheat flour. The method was sought validated for 18 pesticides (appendix 1) in wheat. 13 pesticides were accepted in the validation process. 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. Chromatogram's and calibration curves

The MRM detection of the pesticide residues were parted in windows according to retention times, windows were partly overlapping. In figure 1 examples of chromatogram's are given.

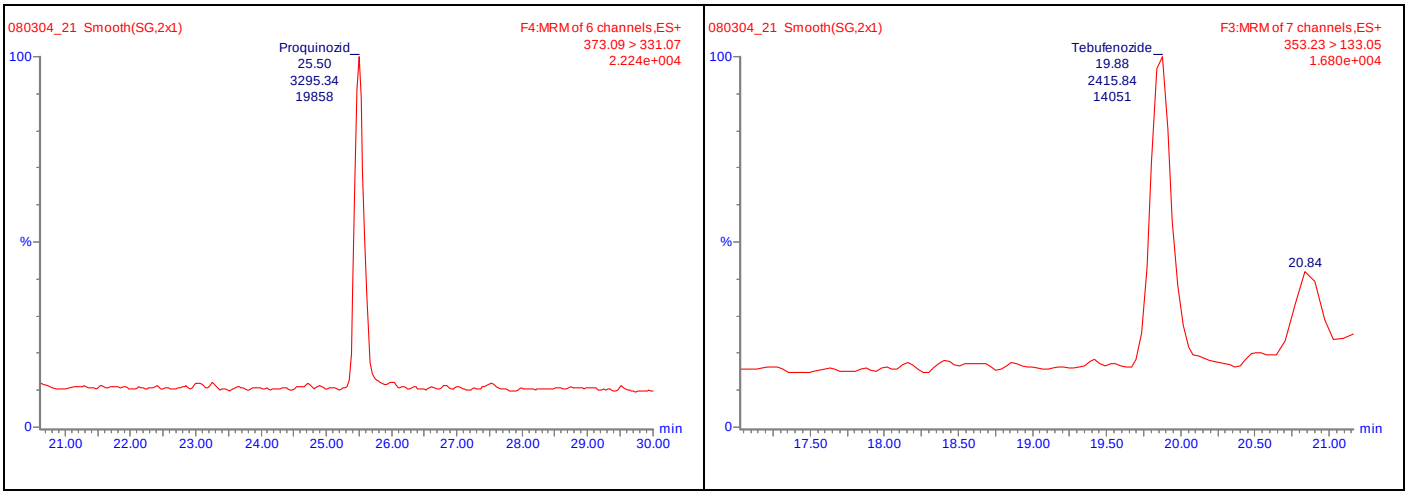

Figure 1. Examples of chromatogram's for proquinazid and tebufenozide at the lowest spiking level (spike concentration 0.011 mg/kg)

The calibration curves were determined by the analysis of each of the 18 pesticides 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.

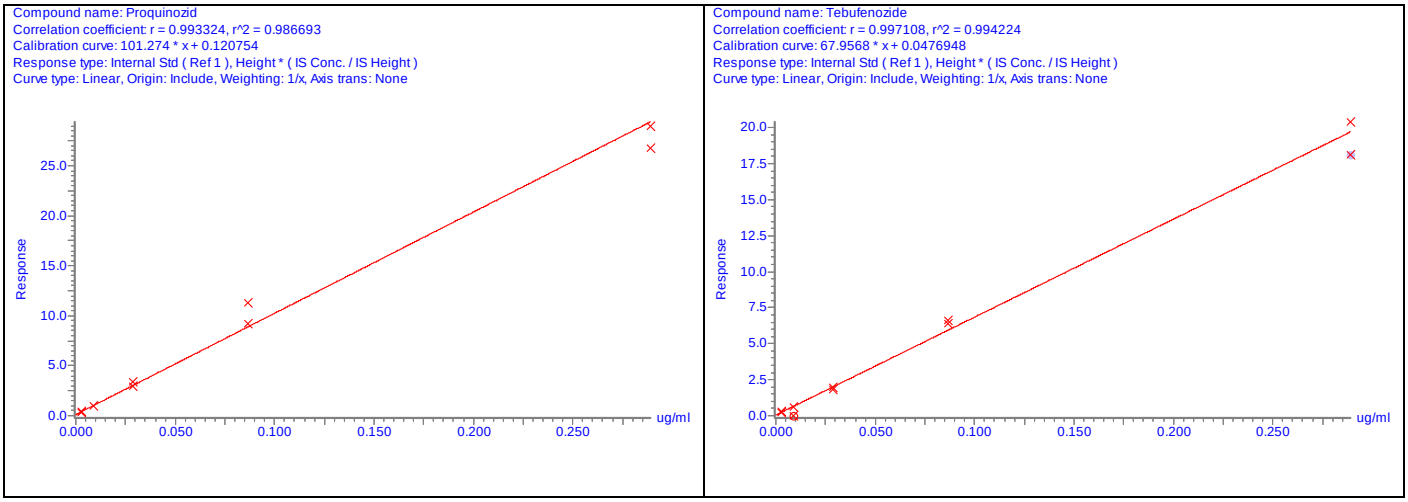

Figure 2. Examples of calibration curves for proquinazid and tebufenozide (concentrations from 0.00289-0.289 µg/ml)

5. Validation parameters

Precision – Repeatability
Repeatability was calculated for all pesticides 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[4].

Appendix 2 shows the relative repeatability for the validated pesticides.

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. For most of the pesticides the recovery from wheat were in the range of 70-110% 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.

Limit of detection, LOQ
LOQ is calculated from the results at the lowest accepted spike level, as 3 times the standard deviation (absolute recovery). The LOQ's 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[5].
2. The average relative recovery must be between 70 and 110%[6].

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

7. Results and discussion

The multi-residue method has been tested for 18 pesticides in wheat flour.

The relative repeatability (RSD_r) varied between 7 to 31 %, however most of the values were around 10-20%. For the majority of the pesticides the recovery was in the range of 60-120% at all three concentration levels.

The criteria for acceptance were met for 13 out of 18 pesticides (listed in Table 1). The LOQs ranged from 0.014 mg/kg to 0.144 mg/kg with a median at 0.019 mg/kg. The lowest calibration level (LCL) was 0.00289 µg/ml corresponding to LOD at 0.006 mg/kg. 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		
Validated (13 compounds)		
Cinidon-ethyl	Flusilazole	Proquinazid
Clodinafoppropargyl	Fonoxaprop-p	Pyraclostrobin
Dimoxystrobin	Metribuzin	Tebufenozide
Famoxadon	Picolinafen	
Flufenacet	Picoxystrobin	
Not accepted (5 compounds)		
Clopyralid	Carbofezin	Thiodicarb
Carboxin	Prothioconazole	

8. Conclusions

In conclusion 13 of 18 pesticides and degradation products were validated.

9. References

1. http://www.quechers.com/
2. The Composition of Foods – fourth edition by Erling Saxholt, Gyldendals, 1996.
3. K. Granby, J. H. Andersen and H.B. Christensen, 2004, Analysis of pesticides in fruit, vegetables and cereals using methanolic extraction and detection by liquid chromatography–tandem mass spectrometry, *Analytica Chemica Acta*, **520**, 165-176
4. 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.
5. W. Horwitz, *Anal. Chem.*, 1982; **54**, 67A.
6. 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.

10. Appendices

Appendix 1. MRM transitions for the pesticides sought validated.

Pesticide/Metabolite	Molecular formula	Molecular weight	mode, ESI +/-	Molecular ion	Transition 1				Transition 2		
					precursor	Product	CV	CE	Product	CV	CE
Carboxin	$C_{12}H_{13}NO_2S$	235.3	ESI+	[M+H]+	236	143	41	15	93	41	30
Cinidon-ethyl	$C_{19}H_{17}Cl_2NO_4$	394.3	ESI+	[M+NH4]+	411	348	10	11	107	10	33
Clodinafoppropargyl	$C_{17}H_{13}ClFNO_4$	349.7	ESI+	[M+H]+	350	266	38	17	91	38	20
Clopyralid	$C_6H_3Cl_2NO_2$	192.0	ESI+	[M+H]+	192	146	55	23	110	52	32
Dimoxystrobin	$C_{19}H_{22}N_2O_3$	326.4	ESI+	[M+H]+	328	116	41	20	206	41	10
Famoxadone	$C_{22}H_{18}N_2O_4$	374.4	ESI+	[M+NH4]+	392	238	38	15	331		
Fenoxaprop-p	$C_{18}H_{16}ClNO_5$	361.8	ESI+	[M+H]+	363	121	25	25	289	25	15
Flufenacet	$C_{14}H_{13}F_4N_3O_2S$	363.4	ESI+	[M+H]+	364	152	56	15	194	56	10
Flusilazole	$C_{16}H_{15}F_2N_3Si$	315.4	ESI+	[M+H]+	316	165	51	20	247	20	17
Flutriafol	$C_{16}H_{13}F_2N_3O$	301.3	ESI+	[M+H]+	303	123	57	25	234	57	20
Metribuzin	$C_8H_{14}N_4OS$	214.3	ESI+	[M+H]+	215	187	52	23	84	21	20
Picolinafen	$C_{19}H_{12}F_4N_2O_2$	376.3	ESI+	[M+H]+	377	256	45	23	238	45	30
Picoxystrobin	$C_{18}H_{16}F_3NO_4$	367.3	ESI+	[M+H]+	369	145	41	25	206	41	10
Proquinazid	$C_{14}H_{17}IN_2O_2$	372.2	ESI +	[M+H]+	373	331	52	11	289	52	25
Prothioconazole	$C_{14}H_{15}Cl_2N_3OS$	344.3	ESI+	[M+H]+	344	189	38	17	125	38	36
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	387.8	ESI+	[M+H]+	388	194	24	11	163	24	25
Tebufenozide	$C_{22}H_{28}N_2O_2$	352.5	ESI +	[M+H]+	353	133	24	17	297	24	5
Thiodicarb	$C_{10}H_{18}N_4O_4S_3$	354.5	ESI +	[M+H]+	355	88	27	15	108	27	15

Appendix 2. Repeatability, recovery and limit of quantification.

In the tables are presented repeatability and LOQ for compounds ionised by ESI+. Values outside the acceptance criteria is marked in italic.

ESI+ Concentration, mg/kg		**Recovery from wheat (%)** 0.011	0.02	0.104	LOQ
Cinidon-ethyl	RSD_r, %			20	0.101
	Recov.,%			80	
Chlodinafoppropargyl	RSD_r, %	31	20	11	0.018
	Recov.,%	89	109	*113*	
Dimoxastrobin	RSD_r, %		18	22	0.023
	Recov.,%		103	99	
Famoxadone	RSD_r, %	21	12	7	0.015
	Recov.,%	102	104	108	
Flufenacet	RSD_r, %	21	21	7	0.014
	Recov.,%	104	*115*	*113*	
Fluzilazole	RSD_r, %		20	10	0.026
	Recov.,%		107	109	
Fenoxaprop-p	RSD_r, %		20	20	0.016
	Recov.,%		*66*	116	
Metribuzin	RSD_r, %			16	0.102
	Recov.,%			95	
Picolinafen	RSD_r, %	26	26	11	0.019
	Recov.,%	109	*121*	*115*	
Picoxystrobin	RSD_r, %			16	0.143
	Recov.,%			*124*	
Proquinazid	RSD_r, %	29	16	14	0.017
	Recov.,%	90	99	97	
Pyraclostrobin	RSD_r, %	28	19	14	0.02
	Recov.,%	107	109	*114*	
Tebufenozide	RSDr, %	25	11	7	0.018
	Recov.,%	110	109	*115*	
Horwitz	RSD, %	32	29	22	