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National Food Institute
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Validation Report 19

Determination of pesticide residues in oat, rye and wheat
by GC-MS/MS and LC-MS/MS

(QuEChERS method)

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December 2015

CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with GC-MS/MS and LC-MS/MS. The method was sought validated for 23 pesticides and metabolites in oat, rye and wheat. The QuEChERS method is an extraction method which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit, vegetables and cereals[1].

2. Principle of analysis

Sample preparation: The samples is milled with a sieve at 1 mm.

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 clean tube and put in -80 degree freezer. When the extract is almost thawed it is centrifuged and the supernatant is transferred to a tube containing PSA and $MgSO_4$. An aliquot was withdrawn prior to this clean-up step and analysed by LC-MS/MS. After shaking and an additional centrifugation step the final extract is diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the matrix matched calibration standards.

Quantification and qualification: The final extracts are analysed by GC/MS/MS and LC-MS/MS. The crude extract withdrawn before PSA clean-up was only analysed by LC-MS/MS.

GC-MS/MS: The pesticide residues are separated on a DB5-MS column and analysed by triple quadrupole operating in the multiple reaction monitoring mode (MRM) with electron energy at 70 eV, source temperature at 180°C and transfer line at 250°C. For each pesticide two sets of precursor and product ions were determined. One for quantification and one for qualification. The MRM transitions for the pesticides and degradation products are given in **Appendix 1a**.

LC-MS/MS: The pesticide residues are separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI). The validation includes pesticides determined in positive mode. $^{13}C_6$-carbaryl was used as internal standard but was not used for the quantification. All pesticides were detected in the MRM mode. For each pesticide or metabolite a precursor ion and 2 product ions 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 1b**.

3. Validation design

The method was sought validated for 23 pesticides or metabolites in oat, rye and wheat, see **Table 1**. The validation was performed on 5-6 replicates on each cereals commodity at each of the three spiking levels; 0.01, 0.02 and 0.1 mg/kg. A blank sample of each cereal commodity was included.

Table 1. Pesticides included in the recovery experiments.

Pesticides included in recovery experiments		
6-Benzylaminopurine	Novaluron	Spirotetramat enol-glucoside
8-Hydroxyquinoline	Penoxsulam	Spirotetramat cis-keto-hydroxy
Amisulbrom	Profoxydim	Spirotetramat mono-hydroxy
Carbetamide	Propaquizafop	Tembotrione
Cyflufenamide	Pyridalyl	Thiencarbazone-methyl
Difenacoum	Quizalofop (free acid)	Triazoxide
Ethirimol	Spinetoram	Triflusulfuron-methyl
Fenpyrazamine	Spirotetramat -Cis-enol	

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels, i.e. 0.003, 0.01, 0.033 and 0.1 µg/ml. The calibration curves were in general best fitted to a linear curve. The quantification was performed from the mean of two bracketing calibration curves. The majority of the correlation coefficients (R) were higher or equal to 0.99. Examples of chromatograms obtained when analysing the extracts by GC-MS/MS are presented in **Figure 1-4**. Examples of calibration curves for LC-MS/MS are presented in **Figure 5-8**.

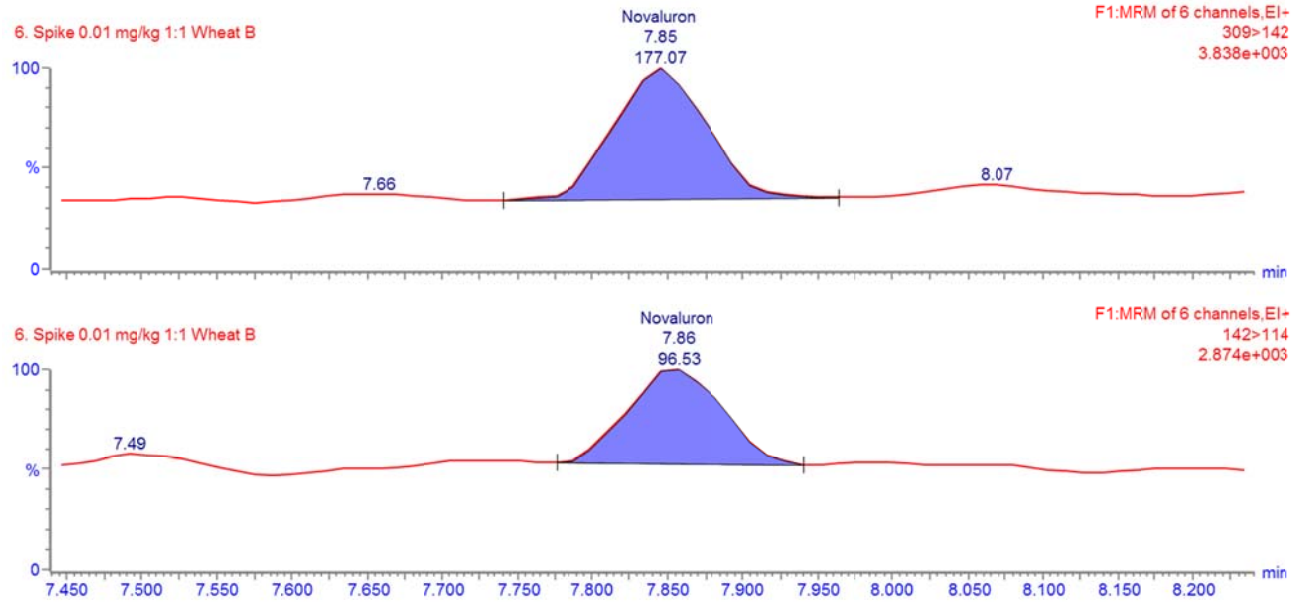

Figure 1: Examples of GC-MS/MS chromatograms for novaluron in wheat obtained when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown for each pesticide).

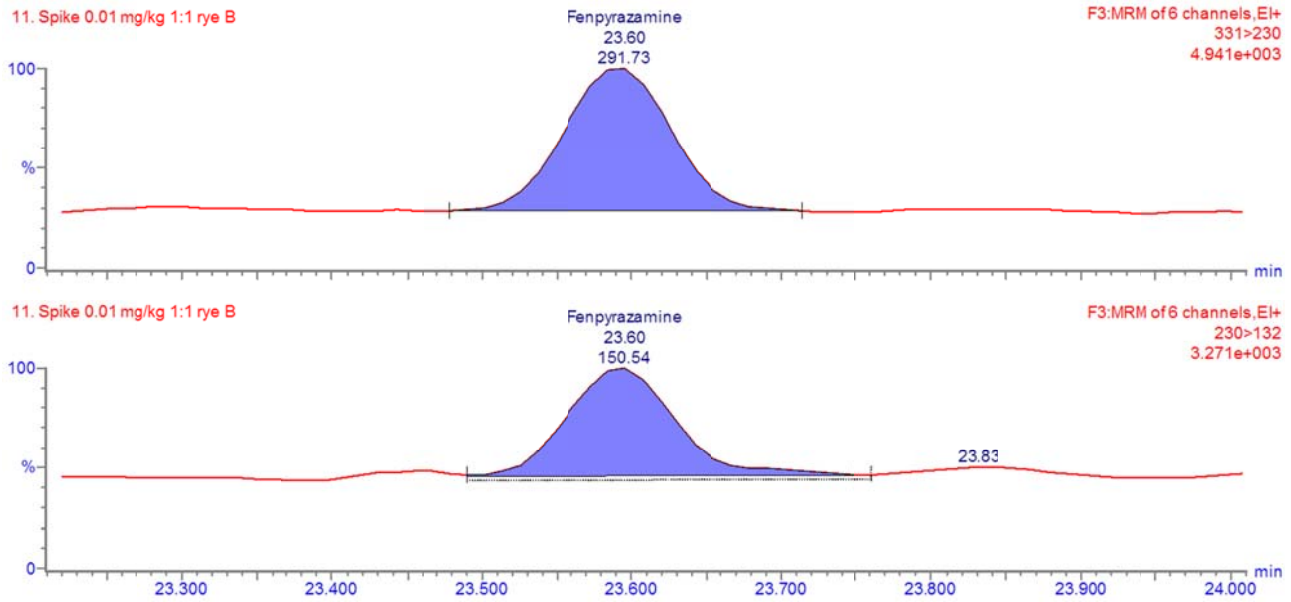

Figure 2: Examples of GC-MS/MS chromatograms fenpyrazamine in rye obtained when analysing extract spiked with 0.01 mg/kg.

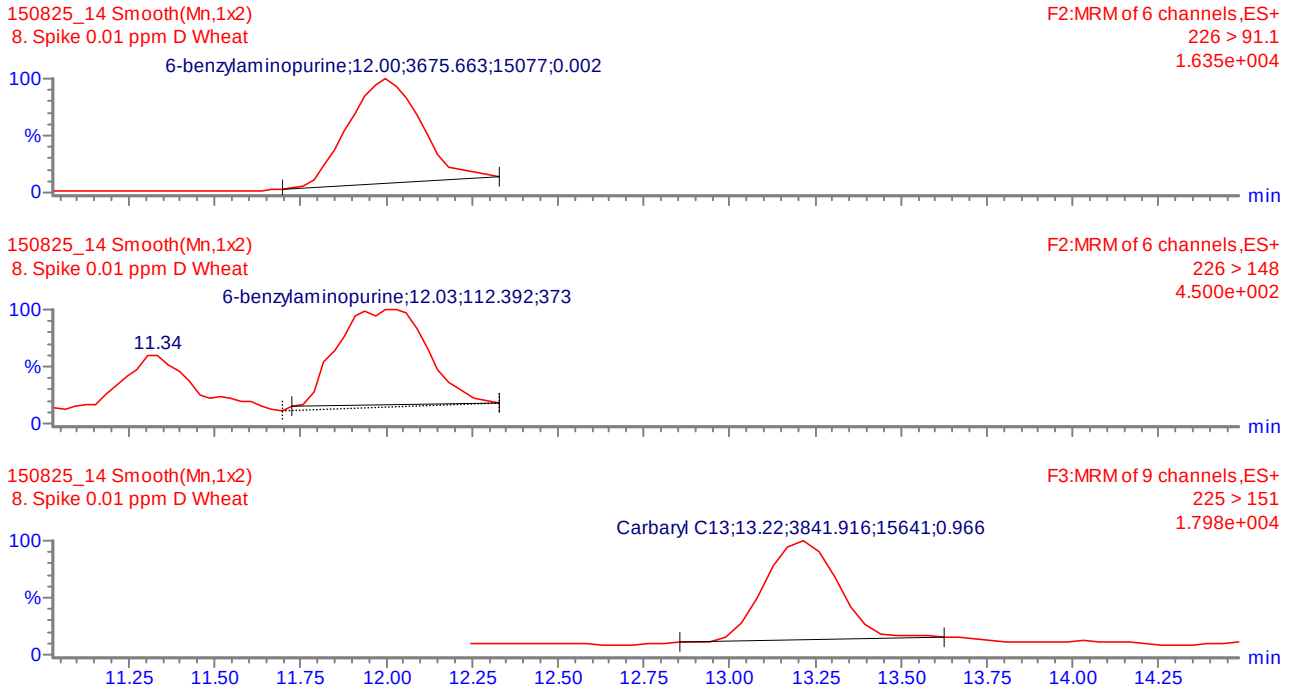

Figure 3: Examples of LC-MS/MS chromatograms 6-benzylaminepurine (6-benzyladenine) in wheat obtained in positive mode when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown for each pesticide).

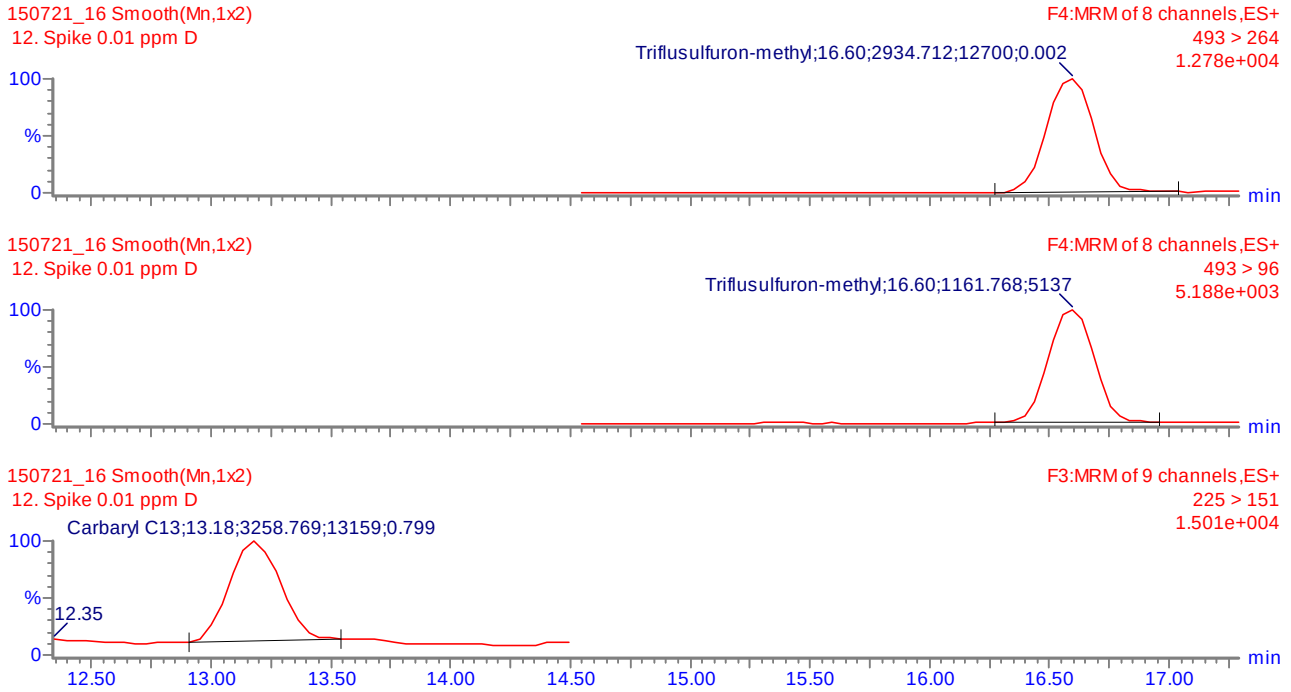

Figure 4: Examples of LC-MS/MS chromatograms triflusulfuron-methyl in rye obtained when analysing extract in positive mode spiked with 0.01 mg/kg.

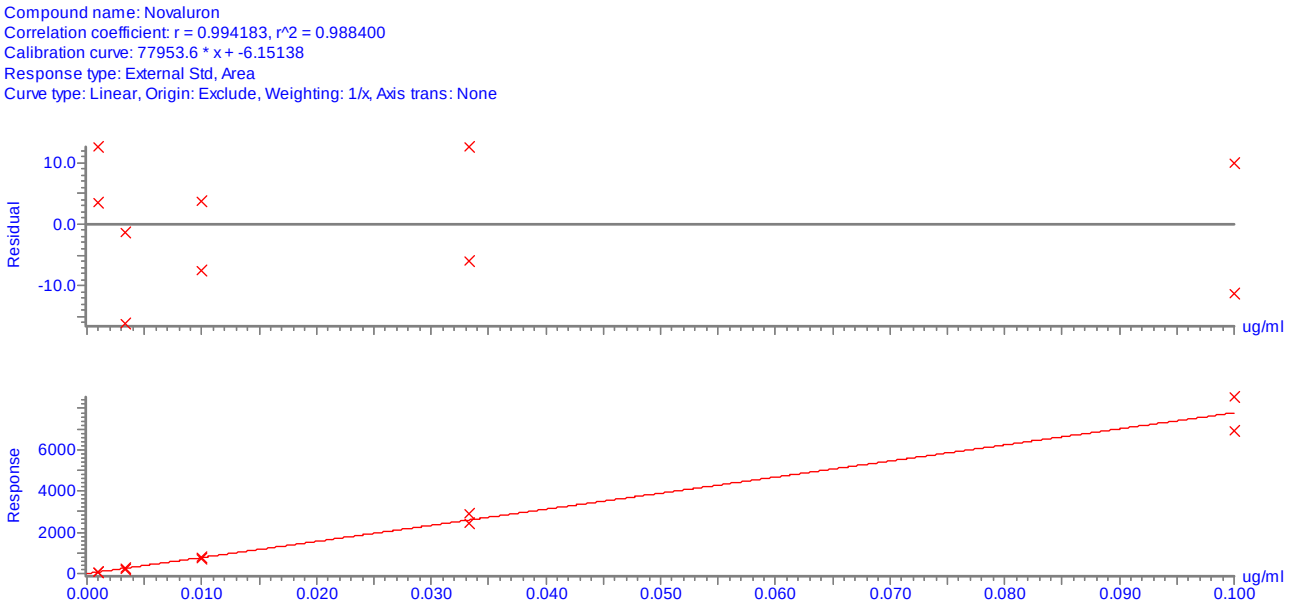

Figure 5. Examples of GC-MS/MS calibration curves for novaluron matrix matched with wheat (concentrations from 0.003-0.100 µg/ml)

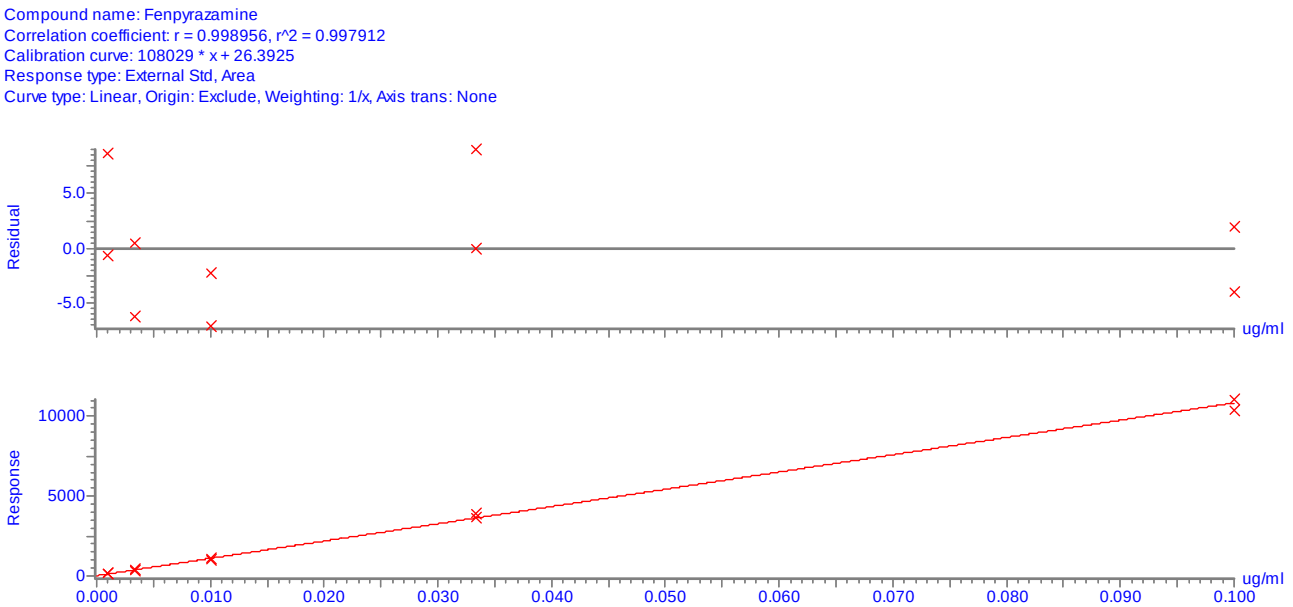

Figure 6. Examples of GC-MS/MS calibration curves for fenpyrazamine matrix matched with rye (concentrations from 0.003-0.100 µg/ml.).

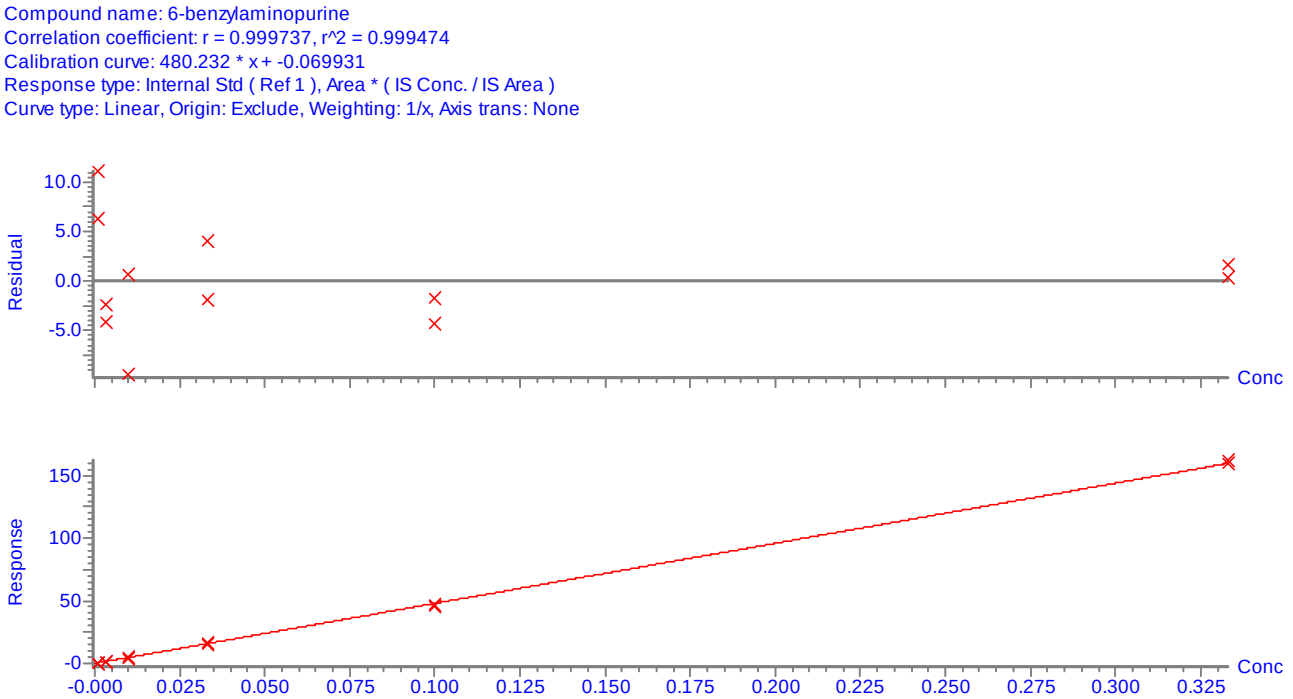

Figure 7. Examples of LC-MS/MS calibration curves for 6-benzylaminepurine (6-benzyladenine) matrix matched with wheat (concentrations from 0.003-0.100µg/ml).

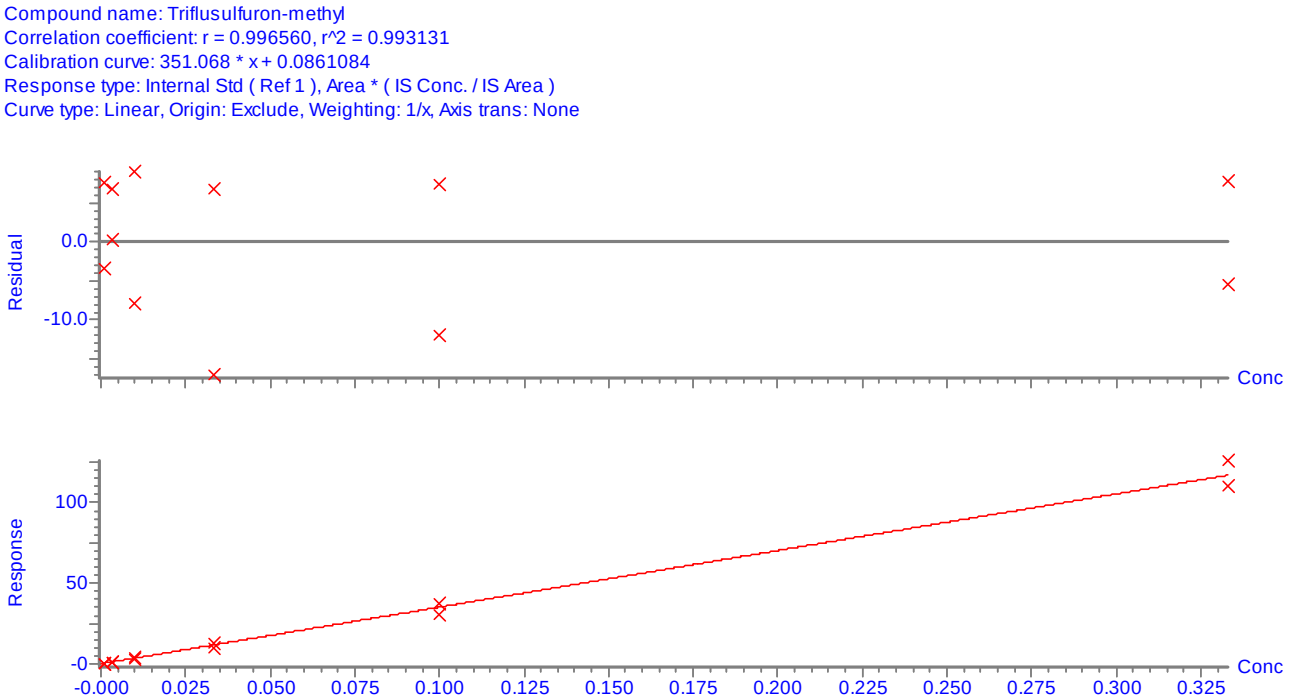

Figure 8. Examples of LC-MS/MS calibration curves for triflusulfuron-methyl matrix match with rye (concentrations from 0.003-0.100µg/ml)

5. Validation parameters

Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides and degradation products on all three spiking levels (0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg), both for the individual cereal commodities and for the all commodities altogether. 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. The internal reproducibility is calculated only for the joint data set including all three cereal commodities, because the individual cereal types were only analysed on one occasion. Internal reproducibility is relative standard deviation on results obtained under reproducibility conditions, with the same method on the same sample by different operators within a larger period of time.

Repeatability (RSD_r) and internal reproducibility (RSD_R) in this validation was calculated from the 5-6 replicate determinations. Repeatability were calculated as given in ISO 5725-2[2].

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg) with the relevant pesticides, isomers and degradation products.

Robustness

The QuEChERS method has, in connection with the development of the method, been shown to be robust by Anastassiades et al. 2003[1].

Limit of quantification, LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria (se Section 6) was meet.

The obtained results including recovery, RSD_r, RSD_R and limit of quantification (LOQ) are presented in appendix 2 for the pooled results obtained for all three types of cereal and in appendix 3 to 5 for the individual cereal types; oat, rye and wheat

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 should be ≤20%[3].
2. The average relative recovery must be between 70 and 120%[3].

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

7. Results and discussion

Overall validation on all 3 cereal types.

Of the 23 compounds included in the validation study (Table 1) 20 compounds were successfully validated on all three cereal types included in the study on LC-MS/MS (10 pesticides) or both LC and GC (10 pesticides), see **Appendix 2**.

For the accepted validation parameters the relative repeatability (RSD_r) varied between 5-22%. The internal reproducibility (RSD_R) also varied between 5-22%. Recoveries was in the range of 67-112% including all three spike levels. The combined LOQs were 0.01 for most compounds though for Difenacoum (LC), Novaluron (GC), Profoxydim (LC), spirotetramat cis-keto-hydroxy (GC) and triflusulfuron-methyl (GC) a LOQ of 0.02 mg/kg was obtained and for amisulbrom (LC), carbetamide (GC) and Pyridalyl (GC) an LOQ of 0.1 was obtained. Recoveries down to 50% was accepted if similar for all three spike levels and RSD_r and RSD_R were relatively low.

Four of the compounds included in the study was not possible to validate. 8-hydroxyquinoline may form complexes with metals and a SRM method may be needed for the quantitative analysis of this analyte. Quizalofop free acid was as expected removed by PSA during the dSPE step. Spirotetramat cis-enol was also removed by PSA.

Validation on individual cereal type.

More or less similar results were obtained if calculating the validation parameters for each of the cereal types individually.

Though for spirotetramat cis-enol an LOQ of 0.01 could be obtained for oat as well as for wheat, though for the latter with a low recovery (53-66%). Though in general are the results obtained for oat the ones that deviate most from the overall validation.

The validation results obtained for the individual cereals types are presented in Appendix 3 (oat), Appendix 4 (rye) and Appendix 5 (wheat). In appendix 6 are the validation results obtained using the same procedures as for the LCMSMS results in Appendix 2 though without inclusion of the dSPE with PSA in the extraction procedure. As can be seen from appendix 6 it is possible to obtain an LOQ of 0.01 mg/kg for 21 compounds including also quizalofop free acid and spirotetramat cis-enol. The only compound showing poorer results when excluding the dSPE step was pyridalyl, for which high RSD_R% was observed with this extraction procedure.

8. Conclusions

In conclusion 20 pesticides were successfully validated on oat, rye and wheat using the QuEChERS method and GC-MS/MS or/and LC-MS/MS. The LOQ obtained were 0.01 mg/kg except for Difenacoum (LC), Novaluron (GC), Profoxydim (LC), spirotetramat cis-keto-hydroxy (GC) and triflusulfuron-methyl (GC) a LOQ of 0.02 mg/kg was obtained and for amisulbrom (LC), carbetamide (GC) and Pyridalyl (GC) a LOQ of 0.1 was obtained. In order to obtain acceptable validation results for quizalofop free acid and spirotetramat cis-enol the dSPE step with PSA needs to be excluded.

9. References

1 EN 15662:2008. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method

2 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

3 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/12495/2011, 01/01/2012, European Commission, Brussels, 2012.

Appendix 1a. MRM transitions GC-MS/MS.

GC-MS/MS	Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CE
8-Hydroxyquinoline	7.8	145	117	10	117	90	10
Amisulbrom	10.53	229	148	10	227	148	15
Carbetamide	14.24	236	119	10	119	91	10
Cyflufenamide	17.77	412	295	5	188	88	20
Fenpyrazamine	23.61	331	230	5	230	132	10
Novaluron	7.84	309	142	10	142	114	10
Propaquizafop	32.76	443	299	15	299	91	10
Pyridalyl	26.98	204	148	15	164	146	10
Spirotetramat cis-keto-hydroxy	24.06	317	128	10	128	96	10
Thiencarbazone-methyl	13.3	124	96	5	218	124	5
Triflusulfuron-methyl	8.86	237	222	10	237	208	10

Appendix 1b. MRM transitions for LC-MS/MS.

LC-MS/MS	Retention time	Precursor ion-1	Product ion-1	CV	CE	Precursor ion-2	Product ion-2	CV	CE
6-Benzylaminopurine	11.93	226	91.1	40	20	226	148	40	20
8-Hydroxyquinoline	8.96	146	118	40	20	146	128	40	20
Amisulbrom	22.05	466	227	50	20	468	148	50	20
Carbetamide	11.73	237	192	30	10	237	118	30	50
Cyflufenamide	20.06	413	241	40	20	413	295	40	10
Difenacoum dobbelt top	22.83	445	179	40	40	445	257	40	10
Ethirimol	13.63	210	98	10	30	210	140	10	20
Fenpyrazamine	17.56	332	230	20	20	332	216	20	30
Novaluron	21.51	493	158	40	20	493	141	40	50
Penoxsulam	13	484	195	10	30	484	164	10	30
Profoxydim (double peak)	21.26/24.19	466	280	50	16	466	180	50	20
Propaquizafop	22.41	444	100	50	20	444	163	50	50
Pyridalyl	28.07	490	109	50	50	492	111	50	20
Quizalofop free acid	17.76	345	299	52	17	345	192	52	20
Spinetoram	23	760.5	142	30	30	760.5	98	30	50
Spinetoram	22.07	748.5	142	30	30	748.5	98	30	50
Spirotetramat -Cis-enol	13.81	302.1	216.1	46	28	302.1	270	46	24
Spirotetramat enol-glucoside	8.66	464.1	302.2	22	12	464.1	216	22	28
Spirotetramat cis-keto-hydroxy	15.16	318.1	300.2	24	16	318.1	268	24	20
Spirotetramat mono-hydroxy	11.83	304.1	254.2	36	20	304.1	131	36	28
Tembotrione	12.74	458	341	40	16	458	262	40	40
Thiencarbazone-methyl	9.78	391	130	50	16	391	359	50	14
Triazoxide	15.3	248	124	50	30	248	150	50	30
Triflusulfuron-methyl	16.67	493	264	50	20	493	96	50	50

Appendix 2. Recoveries, repeatability (RSD_r), internal reproducibility (RSDR) and Limit of Quantification (LOQ) for pesticides validated on three cereal commodities, oat, rye and wheat using QuEChERS.

Numbers in italic is outside 70-120% recovery or above 20% RSD

	Oat, rye and wheat - QuEChERS	Spike level **0.01 mg/kg**				Spike level 0.02 mg/kg				Spike level 0.1 mg/kg				
		Recovery %	**RSD_r, %**	**RSD_R, %**		**Recovery %**	**RSD_r, %**	**RSD_R, %**		**Recovery %**	**RSD_r, %**	**RSD_R, %**		**LOQ**
LC	6-benzylaminopurine	85	6	6		81	8	9		76	7	7		0.01
LC	Amisulbrom	*97*	*40*	*44*		*78*	*38*	*38*		99	12	12		0.1
GC	Amisulbrom	94	16	16		96	13	12		88	9	9		0.01
LC	Carbetamide	112	6	6		104	9	9		94	5	6		0.01
GC	Carbetamide	*104*	*14*	*34*		*109*	*13*	*26*		103	8	12		0.1
LC	Cyflufenamide	109	6	6		107	9	9		100	8	9		0.01
GC	Cyflufenamide	112	8	8		105	5	5		101	5	6		0.01
LC	Difenacoum	*112*	*9*	*29*		99	10	21		94	8	10		0.02
LC	Ethirimol	85	5	9		88	9	10		87	8	9		0.01
LC	Fenpyrazamine	106	7	11		110	9	10		112	8	9		0.01
GC	Fenpyrazamine	107	10	16		108	7	12		102	6	6		0.01
LC	Novaluron	102	10	13		97	13	14		96	11	10		0.01
GC	Novaluron	*87*	*14*	*24*		85	12	18		84	10	16		0.02
LC	Penoxsulam	98	9	8		94	11	11		88	6	7		0.01
LC	Profoxydim	*88*	*26*	*26*		80	14	14		81	9	10		0.02
LC	Propaquizafop	101	5	6		97	10	9		94	9	13		0.01
GC	Propaquizafop	93	8	20		99	9	20		87	7	8		0.01
LC	Pyridalyl	78	12	19		70	9	12		*67*	7	15		0.01
GC	Pyridalyl	*80*	*7*	*32*		*82*	*11*	*24*		70	12	12		0.1
LC	Spinetoram	111	7	9		104	9	10		97	9	10		0.01
LC	Spirotetramat cis-keto-hydroxy	104	8	10		100	10	9		96	8	11		0.01
GC	Spirotetramat cis-keto-hydroxy	*106*	*31*	*29*		110	11	15		91	9	8		0.02
LC	Spirotetramat enol-glucoside	75	11	14		70	13	14		*67*	7	7		0.01

	Oat, rye and wheat - QuEChERS	Spike level **0.01 mg/kg**				Spike level 0.02 mg/kg				Spike level 0.1 mg/kg				
		Recovery %	**RSD_r, %**	**RSD_R, %**		**Recovery %**	**RSD_r, %**	**RSD_R, %**		**Recovery %**	**RSD_r, %**	**RSD_R, %**		**LOQ**
LC	Spirotetramat mono-hydroxy	104	7	9		103	11	12		97	6	8		0.01
LC	Tembotrione	91	15	16		73	13	20		70	7	11		0.01
LC	Thiencarbazone-methyl	107	12	11		100	9	10		95	6	8		0.01
GC	Thiencarbazone-methyl	83	22	22		81	15	14		78	8	11		0.01
LC	Triazoxide	100	18	19		91	11	15		84	8	9		0.01
LC	Triflusulfuron-methyl	99	6	10		94	11	10		91	7	11		0.01
GC	Triflusulfuron-methyl	*87*	*16*	*28*		91	8	11		84	8	7		0.02
Pesticides/metabolites not possible to validate														
LC	8-hydroxyquinoline	e, a, b												
LC	Quizalofop free acid	e												
LC	Spirotetramat cis-enol	e, b												

a) RSDr > 20%; b) RSDR > 20%; c) Not GC-MS/MS amenable; d) Not LC-MS/MS amenable; e) Recovery <50%; f) Recovery >50%; h) not multimetod amenable; j) wheat results not included because not possible to validate for this matrix.

Appendix 3. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQs) for pesticides validated on Oat using QuEChERS.

	Oat - QuEChERS	Spike level, mg/kg 0.01		Spike level, mg/kg 0.02		Spike level, mg/kg 0.1		
		Recovery %	**RSDr %**	**Recovery %**	**RSDr %**	**Recovery %**	**RSDr %**	**LOQ**
LC	6-benzylaminopurine	84	4	85	11	76	6	0.01
LC	Amisulbrom	*66*	*70*	*76*	*54*	93	16	0.1
GC	Amisulbrom	89	16	93	13	91	8	0.01
LC	Carbetamide	112	7	109	12	94	3	0.01
GC	Carbetamide	*58*	*31*	78	22	98	10	0.02
LC	Cyflufenamide	112	6	111	11	99	5	0.01
GC	Cyflufenamide	116	9	102	4	98	4	0.01
LC	Difenacoum	*147*	*3*	120	11	95	10	0.02
LC	Ethirimol	82	4	87	13	83	6	0.01
LC	Fenpyrazamine	108	6	113	12	108	5	0.01
GC	Fenpyrazamine	92	11	96	12	102	5	0.01
LC	Novaluron	108	12	104	18	95	6	0.01
GC	Novaluron	*67*	*14*	72	15	74	5	0.02
LC	Penoxsulam	98	5	99	10	89	6	0.01
LC	Profoxydim	*82*	*31*	83	12	79	6	0.02
LC	Propaquizafop	101	6	96	12	86	5	0.01
GC	Propaquizafop	76	7	82	9	83	5	0.01
LC	Pyridalyl	87	13	*62*	*14*	*61*	*8*	0.01
GC	Pyridalyl	54	3	61	14	66	4	0.01* low recovery
LC	Spinetoram	118	7	110	12	96	6	0.01
LC	Spirotetramat cis-enol	107	7	101	13	83	9	0.01
LC	Spirotetramat cis-keto-hydroxy	95	11	98	14	90	4	0.01
GC	Spirotetramat cis-keto-hydroxy	*111*	*50*	121	8	94	7	0.02
LC	Spirotetramat enol-glucoside	80	13	75	16	*67*	*6*	0.01

	Oat - QuEChERS	Spike level, mg/kg 0.01		Spike level, mg/kg 0.02		Spike level, mg/kg 0.1		
		Recovery %	**RSDr %**	**Recovery %**	**RSDr %**	**Recovery %**	**RSDr %**	**LOQ**
LC	Spirotetramat mono-hydroxy	106	6	108	16	93	3	0.01
LC	Tembotrione	94	11	85	8	76	7	0.01
LC	Thiencarbazone-methyl	109	12	104	11	91	7	0.01
GC	Thiencarbazone-methyl	*92*	*23*	77	19	70	9	0.02
LC	Triazoxide	100	15	97	10	83	6	0.01
LC	Triflusulfuron-methyl	100	6	95	13	83	8	0.01
GC	Triflusulfuron-methyl	78	17	86	5	85	7	0.01
Pesticides/metabolites not possible to validate								
	Please refer to appendix 2.							

*: The recovery is above 120% but the data are accepted due to the low RSDr%.

Appendix 4. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQs) for pesticides validated on rye using QuEChERS.

	Rye - QuEChERS	Spike level, mg/kg 0.01			Spike level, mg/kg 0.02			Spike level, mg/kg 0.1			
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	6-benzylaminopurine	85	9		76	7		76	6		0.01
LC	Amisulbrom	112	7		80	25		105	10		0.01
GC	Amisulbrom	91	12		97	10		87	7		0.01
LC	Carbetamide	115	6		101	9		97	5		0.01
GC	Carbetamide	116	15		117	8		98	6		0.01
LC	Cyflufenamide	106	8		102	8		107	7		0.01
GC	Cyflufenamide	109	10		107	5		105	4		0.01
LC	Difenacoum	93	15		86	11		100	6		0.01
LC	Ethirimol	82	7		84	6		88	5		0.01
LC	Fenpyrazamine	96	11		104	10		119	5		0.01
GC	Fenpyrazamine	109	11		110	5		101	5		0.01
LC	Novaluron	92	8		90	6		98	8		0.01
GC	Novaluron	93	14		92	13		96	10		0.01
LC	Penoxsulam	95	9		94	13		91	5		0.01
LC	Profoxydim	*83*	*35*		75	11		87	8		0.02
LC	Propaquizafop	97	3		96	8		104	7		0.01
GC	Propaquizafop	97	10		95	6		87	9		0.01
LC	Pyridalyl	*64*	*13*		71	7		77	6		0.02
GC	Pyridalyl	83	7		89	11		73	14		0.01
LC	Spinetoram	105	8		99	7		102	5		0.01
LC	Spirotetramat cis-keto-hydroxy	107	7		99	9		104	7		0.01
GC	Spirotetramat cis-keto-hydroxy	97	11		98	5		91	8		0.01
LC	Spirotetramat enol-glucoside	78	8		66	10		67	4		0.01
LC	Spirotetramat mono-hydroxy	96	9		97	9		103	4		0.01

	Rye - QuEChERS	Spike level, mg/kg 0.01			Spike level, mg/kg 0.02			Spike level, mg/kg 0.1			
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	Tembotrione	98	17		75	16		70	5		0.01
LC	Thiencarbazone-methyl	109	13		98	11		100	3		0.01
GC	Thiencarbazone-methyl	79	17		82	13		79	4		0.01
LC	Triazoxide	*89*	*24*		79	14		89	8		0.02
LC	Triflusulfuron-methyl	91	6		91	12		99	5		0.01
GC	Triflusulfuron-methyl	77	13		86	9		85	7		0.01
Pesticides/metabolites not possible to validate											
	Please refer to appendix 2.										

Appendix 5. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQs) for pesticides validated on wheat using QuEChERS.

	Wheat - QuEChERS	Spike level, mg/kg 0.01			Spike level, mg/kg 0.02			Spike level, mg/kg 0.1			
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	6-benzylaminopurine	86	5		81	4		77	9		0.01
LC	Amisulbrom	*107*	*45*		*78*	*33*		99	11		0.1
GC	Amisulbrom	101	19		98	15		86	12		0.01
LC	Carbetamide	109	5		102	3		91	8		0.01
GC	Carbetamide	122	5		127	12		114	8		0.01*
LC	Cyflufenamide	110	4		107	5		96	12		0.01
GC	Cyflufenamide	113	6		104	5		98	6		0.01
LC	Difenacoum	95	8		90	4		88	9		0.01
LC	Ethirimol	93	3		94	4		91	11		0.01
LC	Fenpyrazamine	114	4		113	4		108	12		0.01
GC	Fenpyrazamine	121	8		118	3		104	7		0.01
LC	Novaluron	107	10		98	11		95	16		0.01
GC	Novaluron	100	14		93	6		83	13		0.01
LC	Penoxsulam	101	11		88	7		83	8		0.01
LC	Profoxydim	100	9		82	19		78	12		0.01
LC	Propaquizafop	105	4		100	7		92	12		0.01
GC	Propaquizafop	110	7		118	10		92	6		0.01
LC	Pyridalyl	83	12		75	5		*63*	*8*		0.01
GC	Pyridalyl	104	7		95	7		72	13		0.01
LC	Spinetoram	112	4		102	4		93	14		
LC	Spirotetramat cis-enol	*63*	*13*		*55*	*12*		*53*	*18*		not PSA amenable
LC	Spirotetramat cis-keto-hydroxy	109	5		101	4		94	11		0.01
GC	Spirotetramat cis-keto-hydroxy	110	7		111	16		90	10		0.01
LC	Spirotetramat enol-glucoside	66	11		68	12		67	11		0.01 (low recov.

	Wheat - QuEChERS	Spike level, mg/kg 0.01			Spike level, mg/kg 0.02			Spike level, mg/kg 0.1			
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	Spirotetramat mono-hydroxy	111	6		104	5		94	10		0.01
LC	Tembotrione	81	16		61	14		64	9		0.01
LC	Thiencarbazone-methyl	103	8		97	5		92	8		0.01
GC	Thiencarbazone-methyl	*77*	*25*		86	9		84	10		0.02
LC	Triazoxide	110	14		96	9		81	9		0.01
LC	Triflusulfuron-methyl	107	5		98	6		90	8		0.01
GC	Triflusulfuron-methyl	110	13		100	9		82	9		0.01
Pesticides/metabolites not possible to validate											
	Please refer to appendix 2.										

*: The recovery is above 120% but the data are accepted due to the low RSDr%.

Appendix 6. Recoveries, repeatability (RSD_r), internal reproducibility (RSDR) and Limit of Quantification (LOQ) for pesticides validated on three cereal commodities, oat, rye and wheat using QuEChERS without the dSPE step.

	Oat, rye and wheat – QuEChERS without dSPE	Spike level **0.01 mg/kg**				Spike level 0.02 mg/kg				Spike level 0.1 mg/kg				
		Recovery %	**RSD_r, %**	**RSD_R, %**		**Recovery %**	**RSD_r, %**	**RSD_R, %**		**Recovery %**	**RSD_r, %**	**RSD_R, %**		**LOQ**
LC	6-benzylaminopurine	85	6	6		83	6	9		76	10	15		0.01
LC	Amisulbrom	93	18	19		101	19	20		90	16	16		0.01
LC	Carbetamide	104	6	6		103	5	7		94	11	14		0.01
LC	Cyflufenamide	103	7	9		96	7	9		84	17	16		0.01
LC	Difenacoum	96	11	10		89	9	9		86	17	19		0.01
LC	Ethirimol	91	6	6		91	5	7		89	8	13		0.01
LC	Fenpyrazamine	98	8	10		102	6	7		100	15	17		0.01
LC	Novaluron	106	12	11		96	11	12		85	19	19		0.01
LC	Penoxsulam	109	7	8		103	7	8		93	13	16		0.01
LC	Profoxydim	82	19	20		75	9	9		73	17	20		0.01
LC	Propaquizafop	94	10	10		90	7	9		83	16	20		0.01
LC	Quizalofop free acid	107	8	9		97	8	10		87	15	15		0.01
LC	Spinetoram	98	7	11		89	6	10		82	13	14		0.01
LC	Spirotetramat cis-enol	119	7	7		106	5	12		94	12	14		0.01
LC	Spirotetramat cis-keto-hydroxy	105	7	7		97	8	10		90	12	15		0.01
LC	Spirotetramat enol-glucoside	90	10	12		83	8	8		81	10	11		0.01
LC	Spirotetramat mono-hydroxy	105	6	6		100	6	8		89	10	13		0.01
LC	Tembotrione	111	12	11		103	10	10		92	14	15		0.01
LC	Thiencarbazone-methyl	107	8	7		102	8	10		89	12	17		0.01
LC	Triazoxide	90	18	17		81	16	15		76	13	15		0.01
LC	Triflusulfuron-methyl	111	7	6		108	5	9		95	15	18		0.01
Pesticides/metabolites not possible to validate														
LC	8-hydroxyquinoline	e, a, b												
LC	Pyridalyl	e, a, b												

a) RSDr > 20%; b) RSDR > 20%; c) Not GC-MS/MS amenable; d) Not LC-MS/MS amenable; e) Recovery <50%; f) Recovery >50%; g) To low sensitivity; h) not multimetod amenable; i) interfering matrix enables quantification.

Appendix 7: Principles of the QuEChERS method for cereal extraction

QuEChERS for cereals (FP417)

Weigh 5 g (±0.05 g) of flour into a 50 ml single use centrifuge tube (red cap). Add internal standard and/or spike standard (maximum 25 µl)

Add a ceramic homogenizer and 10 g of cold water and shake briefly

Add 10 ml acetonitrile and shake vigorously by hand for 1 min. (1. extraction)

Add the prepared mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3 citrate dihydrate and 0.5 g Na_2H cirate sesquihydrate. Shake for a few seconds after each addition to prevent lumps.

Shake vigorously for 1 min. (2. Extraction with phase separation)

Centrifuge for 10 min at 4500 rpm

Transfer at least 8 ml of the extract to a 15 ml single use centrifuge tube and store in the freezer (-80˚C for 1 hour or over night). When the extract are almost thawed (i.e. About -40 ˚C) centrifugate (should be cold 5 C) for 5 min. at 4500 rpm.

Transfer 6 ml of the cold extract to a 15 ml single use centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$. Close the tube and shake vigorously for 30 seconds.

Centrifuge for 5 min. at 4500 rpm

Transfer 4 ml of the extract to a 15 ml single use centrifuge tube. Add 40 µl of 5% formic acid solution in acetonitrile (10 µl/ml extract). Dilute the extract 1:1 with acetonitrile

Transfer the final extract into auto sampler vials and analyse by GC and LC.