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National Food Institute
Technical University of Denmark

Validation Report 16

Determination of pesticide residues in wheat, barley and rice by GC-MS/MS and LC-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 and LC-MS/MS. The method was sought validated for 34 pesticides in wheat, rice and barley. 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 $MgSO_4$. 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 extract is analysed by GC/MS/MS and 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. The injection volume was 4 µl. 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 with both positive and negative ESI. $^{13}C_6$-carbaryl was used as internal standard but was not used for the quantification. All pesticides were detected in the MRM mode. For each pesticide 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 south validated for 34 pesticides or degradation products in wheat, rice and barley, 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		
2-hydroxypropoxycarbazone	Dichlobenil	Isocarbophos
4-trifluoromethylnicotinic acid (TNFA)	Dimethenamid	Meptyldinocap
Acibenzolar-S-Methyl	Ethalfluralin	Metobromuron
Anthraquinone	Ethofumesate	Nicosulfuron
Asulam	Ethoxysulfuron	Propachlor
AvermectinB1a (Abamectin)	Etoxazole	Propoxycarbazone-sodiam salt
Beflubutamid	Fenpyroximate	Pyraflufen-Ethyl
Bifenazate	Flumioxazin	Rotenone
Biphenyl	Foramsulfuron	Spirotetramat
Butralin	Forchlorfenuron	Tetramethrin
Carfentrazone-Ethyl	Imazosulfuron	Trinexapac-ethyl
Chlorotoluron		

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**.

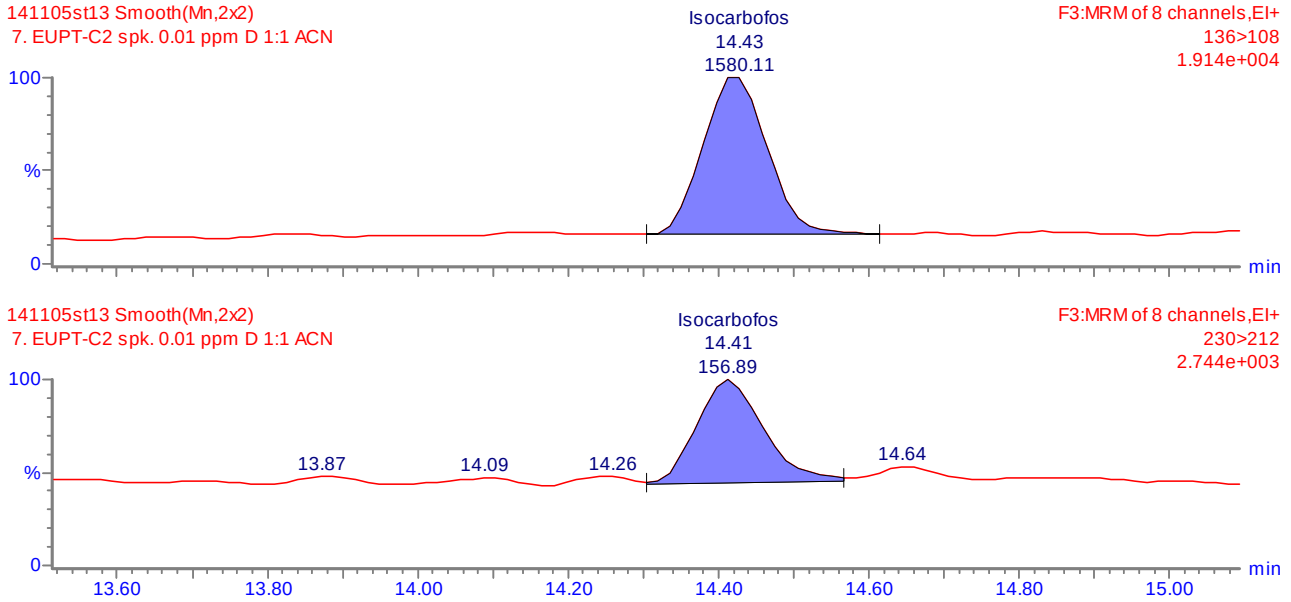

Figure 1: Examples of GC-MS/MS chromatograms for isocarbophos in wheat obtained when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown for each pesticide).

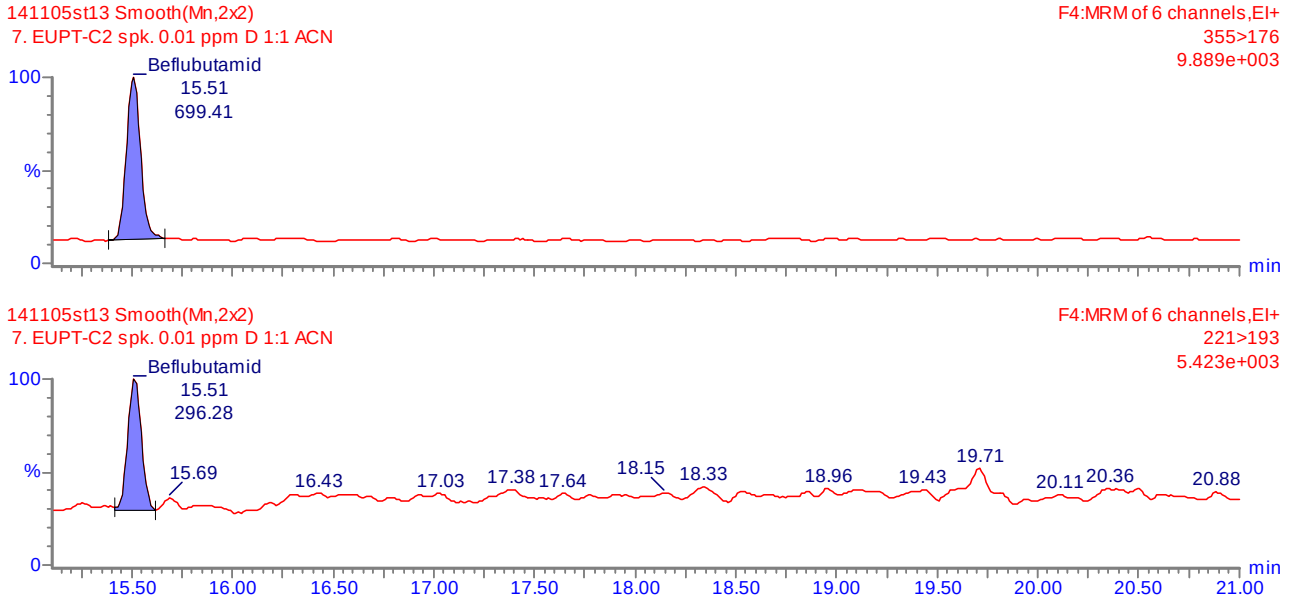

Figure 2: Examples of GC-MS/MS chromatograms beflubutamid obtained when analysing extract spiked with 0.01 mg/kg.

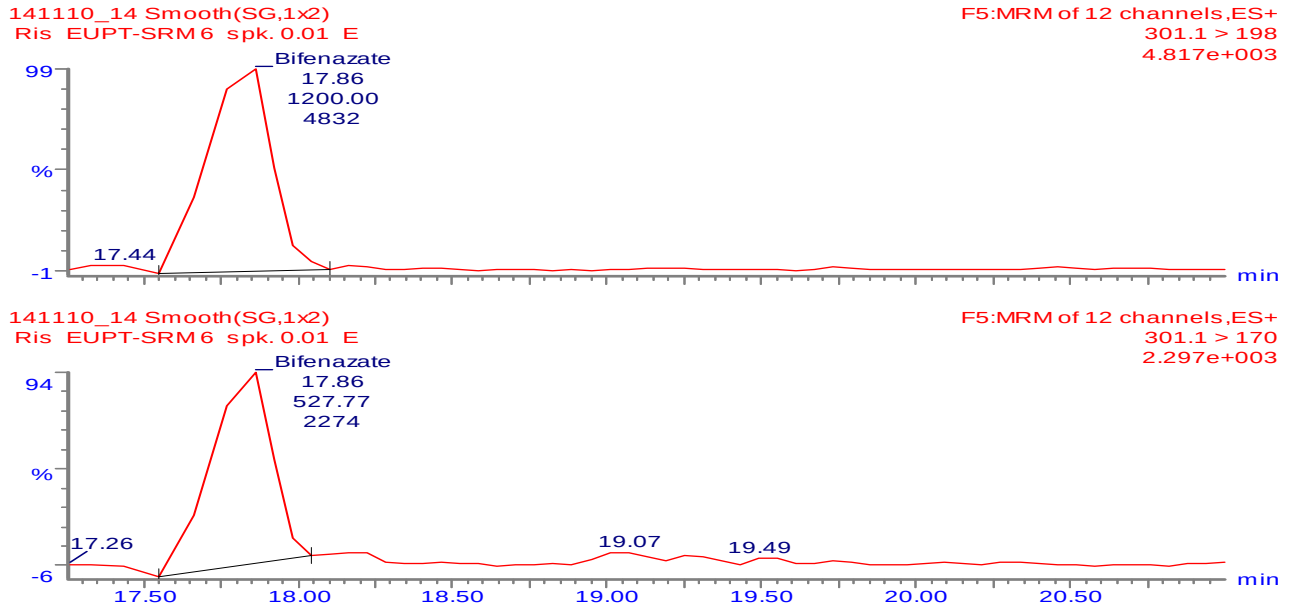

Figure 3: Examples of LC-MS/MS chromatograms bifenazate in rice obtained in positive mode when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown for each pesticide).

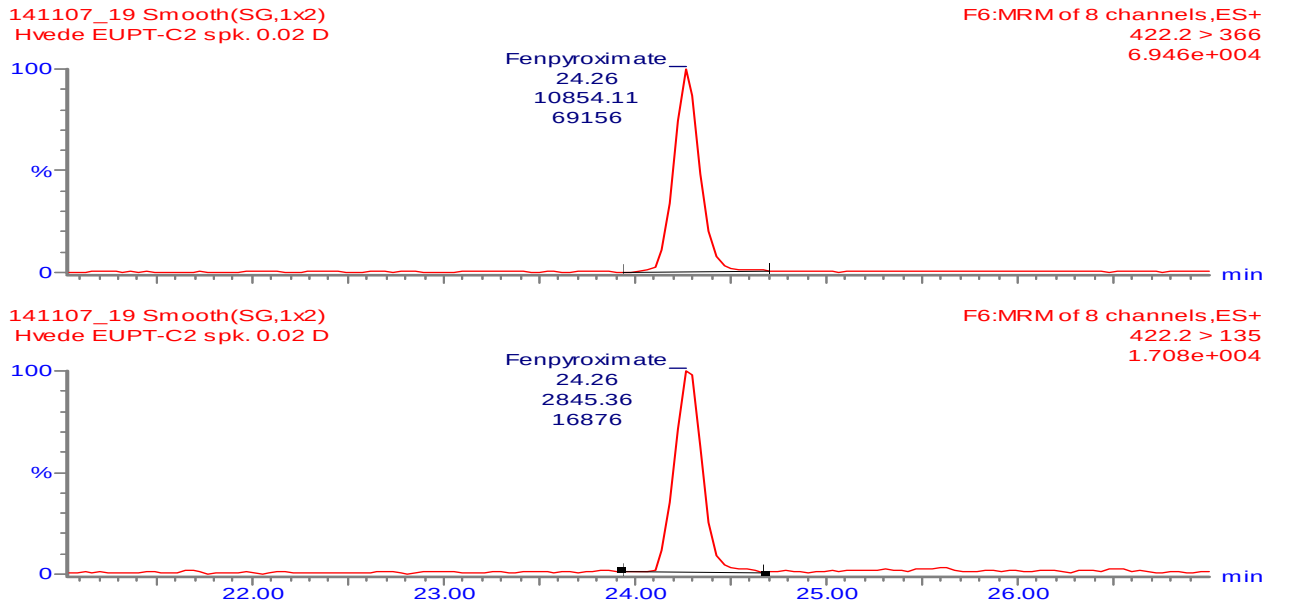

Figure 4: Examples of LC-MS/MS chromatograms fenpyroximate in rice obtained when analysing extract in positive mode spiked with 0.02 mg/kg.

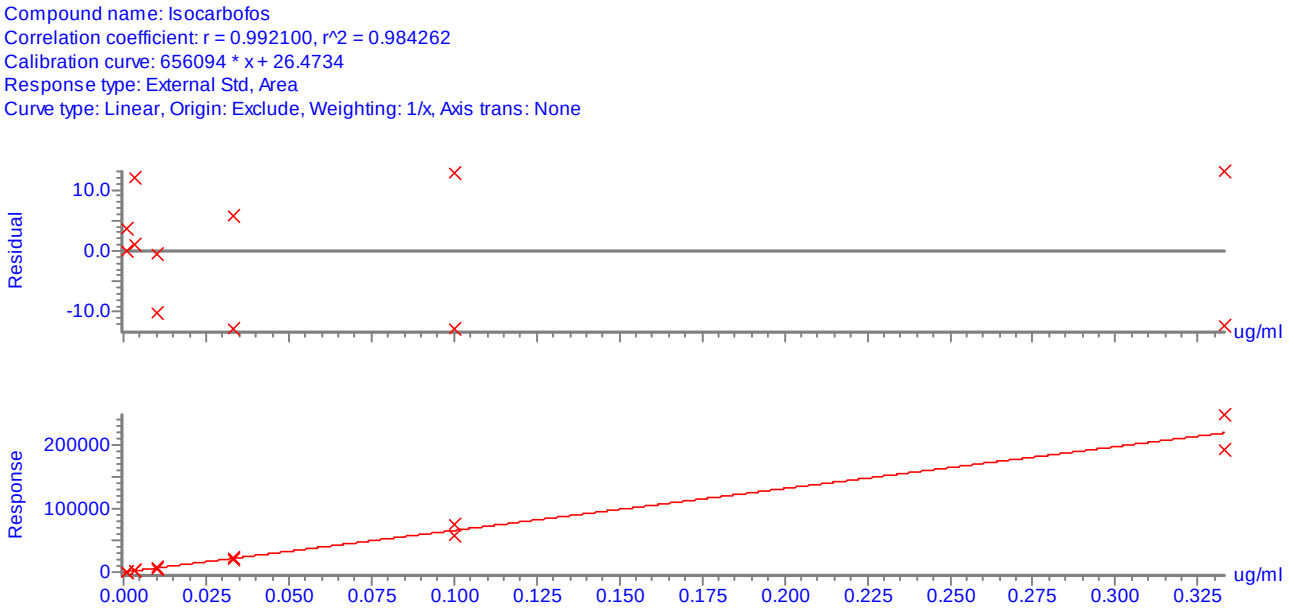

Figure 5. Examples of GC-MS/MS calibration curves for isocarbophos matrix matched with wheat (concentrations from 0.001-0.333 µg/ml)

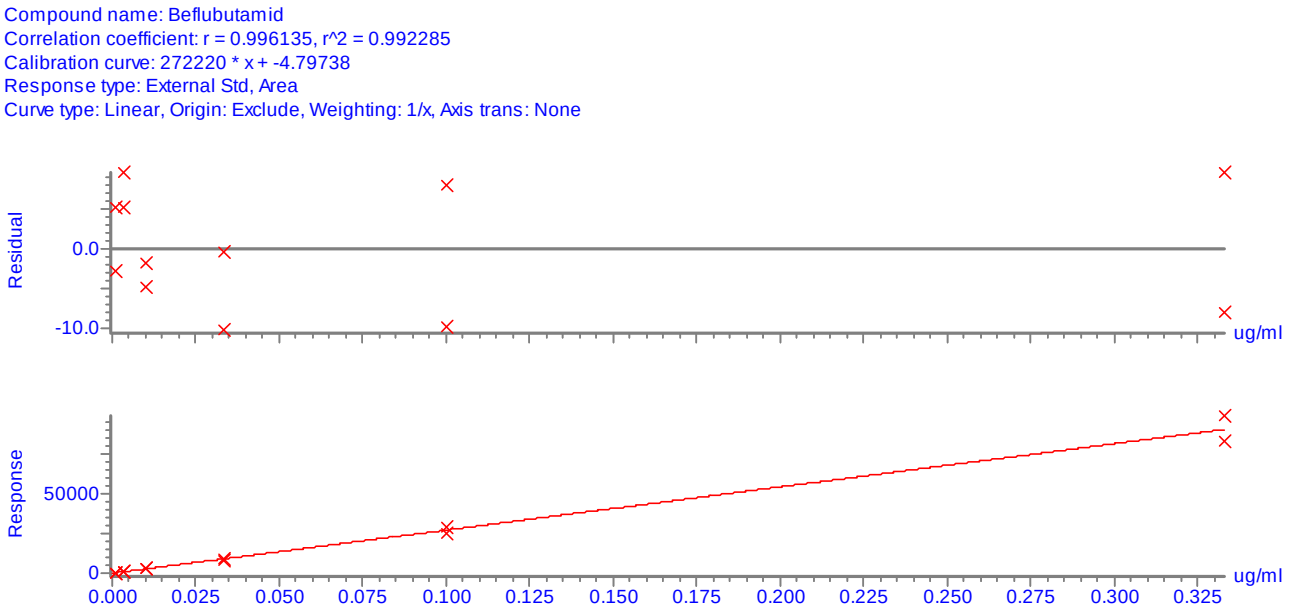

Figure 6. Examples of GC-MS/MS calibration curves for beflubutamid matrix matched with wheat (concentrations from 0.001-0.333 µg/ml.).

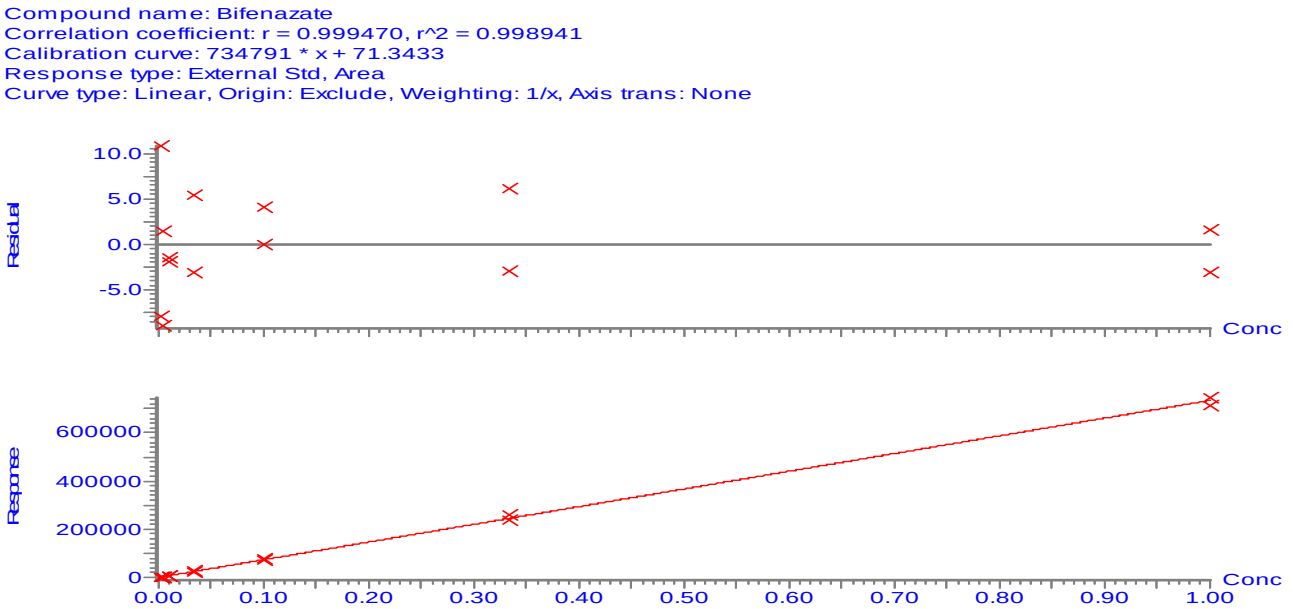

Figure 7. Examples of LC-MS/MS calibration curves for bifenazate matrix matched with wheat (concentrations from 0.001-1.0 µg/ml).

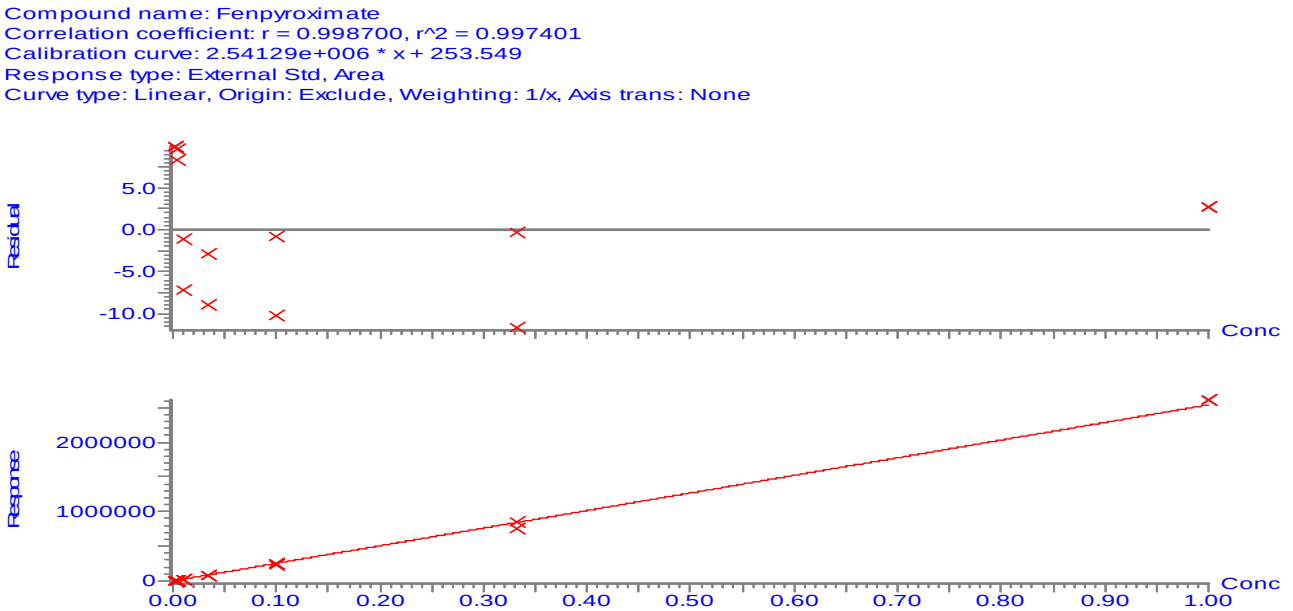

Figure 8. Examples of LC-MS/MS calibration curves for fenpyroximate matrix match with rice (concentrations from 0.001-1.0 µg/ml)

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 for the all the cereal commodities only, because the individual cereal type is analysed on one occasion only. 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; wheat, barley and rice

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 $\leq 20\%$[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

Initially acibenzolar acid, chloropicrin and dithianon were to be included in the recovery study, though it was not possible to find precursor and product ions for the two former compounds and dithianon was found to be lost during the extraction/clean-up procedure. Acibenzolar acid has though been reported in the literature to be possible to analyse by LC-MS/MS in negative ionization mode. However as it is an acid clean-up using PSA should probably be avoided. Chloropicrin has also by others been reported to be difficult to analyse by both GC-MS/MS and LC-MS/MS. Dithianon is sensitive to heat and may therefore be degraded during the extraction because of the increased temperatures resulting from the addition of $MgSO_4$.

Overall validation on all 3 cereal types.

Of the 34 compounds included in the validation study (Table 1) 31 compounds were successfully validated on all three cereal types analysed by GC-MS/MS (10 pesticides), LC-MS/MS (14 pesticides) or both (7 pesticides), see **Appendix 2**.

For the accepted validation parameters the relative repeatability (RSD_r) varied between 3-18 % with an average on 8%. The internal reproducibility (RSD_R) varied between 5-23% with an average on 12%. Recoveries was in the range of 50-123% at all three concentration levels with an average on 88%. The combined LOQs were in the range of 0.01-0.02 mg/kg. Recoveries down to 50% was accepted if similar for all three spike levels and RSD_r and RSD_R were relatively low.

Asulam, foramsulfuron and TFNA were not possible to validate neither on GC-MS/MS or LC-MS/MS. For asulam the recovery were low (<50%) and at the two lowest spike levels were RSD_r and RSD_R >20%. For foramsulfuron low recoveries were found, though the recovery was equally low for all three spike levels and RSD_r and RSD_R were <20%. Foramsulfuron belong to the group of sulfonylureas and most of these are unstable at low pH and may not be protected sufficiently at the pH of the extract. Further if extract are stored for several days during analysis and perhaps reanalysis the compound may be degraded. Foramsulfuron is also a weak acid why some may be bound to PSA during d-SPE. TFNA resulted in an insufficient response by LC-MS/MS to produce data useful for validation and were not GC-MS/MS amenable. TFNA which is also an acid is most likely bound to PSA during the d-SPE. TFNA may also on some instruments give a better signal in ESI negative mode (for more information refer to : http://www.crl-pesticides.eu/userfiles/file/EurlSRM/EurlSRM_meth_FlonicamidMetabolites.pdf

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 an LOQ of 0.1 could be obtained for metobromuron if only including the data obtained for one cereal type whereas it was not possible to validate in the overall validation due to RSD_R>20%.

The validation results obtained for the individual cereals types are presented in Appendix 3 (wheat), 4 (barley) and 5 (rice).

8. Conclusions

In conclusion 31 pesticides were successfully validated on wheat, barley and rice using the QuEChERS method and GC-MS/MS or/and LC-MS/MS. The LOQ obtained were 0.01 mg/kg except for three pesticides for which an LOQ of 0.02 mg/kg was obtained.

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
Acibenzolar-S-Methyl	13.1	182	181	5	182	153	22
Anthraquinone	14.1	180	152	15	208	180	10
Beflubutamid	15.5	221	193	10	355	176	10
Bifenazate	21.5	300	258	10	300	196	15
Biphenyl	8.0	153	152	15	154	153	15
Butralin	14.7	266	220	15	266	190	15
Carfentrazone-Ethyl	19.4	411	340	10	411	330	10
Dichlobenil	7.8	173	138	15	171	136	15
Dimethenamid	12.7	232	154	10	230	154	10
Ethalfluralin	10.0	276	202	10	316	276	10
Ethofumesate	13.7	207	161	10	286	207	12
Etoxazole	21.8	300	270	22	302	274	15
Flumioxazin	28.2	354	326	10	354	312	10
Isocarbophos	14.4	230	212	10	136	108	15
Propachlor	9.7	196	120	10	176	120	10
Pyraflufen-Ethyl	19.9	412	349	15	349	307	15
Tetramethrin	21.5	164	135	10	164	107	17

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
2-hydroxypropoxy-carbazone	8.1	415.1	174	40	12	415.1	116	40	10
4-trifluoromethyl-nicotinic acid (TNFA)	6.7	192.0	78.8	40	30	192.0	97.9	40	26
Asulam	7.3	230.9	156	40	20	230.9	92	40	20
AvermectinB1a	25.5	890.6	567.4	40	10	890.6	305.1	40	30
Butralin	24.0	296.0	240	30	12	296.0	222	30	20
Carfentrazone-Ethyl	19.3	412.2	346	40	20	412.2	366	40	17
Chlorotoluron	14.3	213.0	72	20	20	213.0	140	20	30
Dimethenamid	16.4	276.0	244	40	10	276.0	168	40	20
Ethofumesate	16.3	287.0	258.9	30	12	287.0	162.2	30	16
Ethoxysulfuron	17.1	399.1	261	40	16	399.1	218	40	28
Fenpyroximate (E) (Z)	24.5	422.2	366	50	10	422.2	135	50	30
Foramsulfuron	13.4	453.2	182	20	23	453.2	254	20	20
Forchlorfenuron	15.6	248.0	129	40	18	248.0	155	40	14
Imazosulfuron	16.4	413.0	156	30	20	413.0	153	30	10
Meptyldinocap (ESI-)	24.8	295.2	192.8	20	40	295.2	163	20	40
Metobromuron	14.5	258.9	170	40	30	258.9	148	40	20
Nicosulfuron	12.0	411.1	182	30	20	411.1	212.9	30	20
Propachlor	14.5	211.9	170	30	10	211.9	94	30	27
Propoxycarbazone-sodium salt	9.9	421.0	180	20	10	421.0	137.9	20	27
Pyraflufen-Ethyl	19.7	413.0	338.9	30	17	413.0	289	30	30
Rotenone	18.9	395.1	213	20	26	395.1	192	20	26
Spirotetramat	17.8	374.2	330.2	20	10	374.2	302.2	20	20
Tetramethrin	22.3	332.2	164	40	23	332.2	135	40	17
Trinexapac-ethyl	14.8	253.0	207	20	12	253.0	69	20	12

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

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

	Wheat, barley and rice - 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	2-hydroxypropoxycarbazone	71	13	16		*68*	9	8		*67*	6	8		0.01
GC	Acibenzolar-S-methyl	91	7	*23*		94	4	18		95	3	14		0.01
GC	Anthraquinone	88	7	11		96	5	13		93	6	12		0.01
LC	Avermectin B1a	99	16	15		102	16	16		96	10	11		0.01
GC	Beflubutamid	90	7	8		99	4	9		100	4	9		0.01
GC	Bifenazate	75	7	18		73	6	14		72	4	10		0.01
GC	Biphenyl		a, b, f			*123*	9	11		99	6	13		0.02
LC	Butralin	78	18	16		81	11	12		93	6	10		0.01
GC	Butralin	90	10	*22*		88	5	16		87	6	15		0.01
LC	Carfentrazone-ethyl	75	15	*23*		88	15	21		96	7	10		0.01
GC	Carfentrazone-ethyl	83	5	14		90	4	12		95	4	8		0.01
LC	Chlorotoluron	89	8	9		88	5	11		88	4	10		0.01
GC	Dichlobenil	98	9	16		102	6	10		102	5	13		0.01
LC	Dimethenamid	88	6	11		87	3	9		90	4	5		0.01
GC	Dimethenamid	94	5	12		99	6	12		97	5	11		0.01
GC	Ethalfluralin	95	12	12		96	13	15		97	9	12		0.01
LC	Ethofumesate	79	15	15		91	5	6		94	5	6		0.01
GC	Ethofumesate	94	8	7		103	7	7		103	5	6		0.01
LC	Ethoxysulfuron	75	9	16		75	6	13		80	5	8		0.01
GC	Etoxazole	92	8	14		94	7	12		95	5	11		0.01
LC	Fenpyroximate	86	6	11		90	4	10		95	5	8		0.01
GC	Flumioxazin	93	9	21		98	7	15		96	6	10		0.01
LC	Forchlorfenuron	77	13	*23*		75	7	13		80	5	7		0.01

	Wheat, barley and rice - 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	Imazosulfuron	*68*	12	12		71	7	8		74	6	7		0.01
GC	Isocarbophos	96	9	*22*		104	8	18		101	4	15		0.01
LC	Meptyldinocap		a, b, j			95	17	16		102	14	13		0.02
LC	Metobromuron	83	14	*22*		82	13	18		85	5	9		0.01
LC	Nicosulfuron	*58*	10	11		*55*	6	9		*55*	7	8		0.01
LC	Propachlor	86	8	11		90	4	9		93	4	10		0.01
GC	Propachlor		b			98	10	10		98	7	11		0.02
LC	Propoxycarbazone sodium salt	81	5	8		79	7	7		80	5	6		0.01
LC	Pyraflufen-ethyl	82	7	8		86	4	11		91	4	11		0.01
GC	Pyraflufen-ethyl	88	6	9		96	5	7		99	4	7		0.01
LC	Rotenone	85	11	13		90	6	12		93	9	13		0.01
LC	Spirotetramat	79	8	12		85	4	11		91	4	8		0.01
LC	Tetramethrin	86	8	8		91	5	10		98	5	10		0.01
GC	Tetramethrin	95	13	20		102	8	16		95	6	12		0.01
LC	Trineexpac-ethyl	*58*	13	17		*52*	14	19		*50*	6	12		0.01
Pesticides/metabolites not possible to validate														
LC	Asulam		a, b, e											
LC	Foramsulfuron		e											
LC	TFNA		h											

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 wheat.

	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	2-hydroxypropoxycarbazone	63	16	68	15	63	8	0.01
GC	Acibenzolar-S-methyl	104	2	105	5	105	3	0.01
GC	Anthraquinone	96	5	106	4	101	9	0.01
LC	Avermectin B1a	96	15	111	12	102	9	0.01
GC	Beflubutamid	95	8	107	6	104	4	0.01
GC	Bifenazate	82	8	81	5	80	3	0.01
GC	Biphenyl	91	*23*	113	7	112	7	0.02
GC	Butralin	95	7	93	5	93	5	0.01
LC	Butralin	78	9	82	13	86	7	0.01
LC	Carfentrazone ethyl	65	18	71	18	87	8	0.01
GC	Carfentrazone-ethyl	73	4	81	3	94	6	0.01
LC	Chlorotoluron	90	9	89	2	84	4	0.01
GC	Dichlobenil	101	8	111	4	116	6	0.01
GC	Dimethenamid	106	5	110	5	107	6	0.01
LC	Dimethenamid	89	6	90	2	91	5	0.01
GC	Ethalfluralin	89	16	89	17	98	14	0.01
GC	Ethofumesate	92	5	106	6	105	7	0.01
LC	Ethofumesate	83	15	94	5	97	5	0.01
LC	Ethoxysulfuron	85	7	85	5	86	6	0.01
GC	Etoxazole	101	8	102	7	101	7	0.01
LC	Fenpyroximate	90	5	90	4	92	6	0.01
GC	Flumioxazin	107	3	111	4	105	8	0.01
LC	Foramsulfuron	52	16	49	13	47	9	0.01
LC	Forchlorfenuron	82	10	80	4	84	6	0.01
LC	Imazosulfuron	68	11	73	11	74	7	0.01

	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**
GC	Isocarbophos	101	9	104	6	103	4	0.01
GC	Metobromuron	a, e, g		a, e, g		116	7	0.1
LC	Metobromuron	78	12	85	6	91	5	0.01
LC	Nicosulfuron	60	11	58	2	54	9	0.01
GC	Propachlor	87	11	98	5	108	6	0.01
LC	Propachlor	88	7	89	4	87	4	0.01
LC	Propoxycarbazone sodium salt	87	4	79	6	76	7	0.01
LC	Pyraflufen ethyl	81	9	79	3	81	5	0.01
GC	Pyraflufen-ethyl	86	6	97	3	103	5	0.01
LC	Rotenone	a		a		82	16	0.1
LC	Spirotetramat	73	8	79	3	86	5	0.01
GC	Tetramethrin	105	9	111	4	105	7	0.01
LC	Tetramethrin	87	10	88	5	91	5	0.01
Pesticides/metabolites not possible to validate								
LC	Asulam	a, e						
LC	Bifenazate	e						
LC	Meptyldinocap	i						
LC	TFNA	h						
LC	Trineexpac-ethyl	e						

a) RSDr > 20%; e) Recovery <50%; g) To low sensitivity; h) not multimetod amenable ; i) interfering matrix enables quantification.

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

	Barley - 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	2-hydroxypropoxycarbazone	78	15		66	4		67	5		0.01
GC	Acibenzolar-S-methyl	67	8		75	6		80	3		0.01
GC	Anthraquinone	79	9		83	7		82	5		0.01
LC	Asulam	70	19		62	12		55	10		0.01
LC	Avermectin B1a	a			62	16		93	5		0.02
GC	Beflubutamid	85	6		92	4		91	5		0.01
GC	Bifenazate	61	8		62	7		68	5		0.01
GC	Biphenyl	*212*	*22*		*132*	9		88	6		0.02
GC	Butralin	69	17		73	7		73	8		0.01
LC	Butralin	a			75	15		91	6		0.02
LC	Carfentrazone ethyl	a			96	20		99	6		0.02
GC	Carfentrazone-ethyl	81	5		89	5		88	5		0.01
LC	Chlorotoluron	8	9		81	4		82	4		0.01
GC	Dichlobenil	83	8		94	10		92	6		0.01
GC	Dimethenamid	85	6		89	11		87	6		0.01
LC	Dimethenamid	79	8		79	5		87	3		0.01
GC	Ethalfluralin	97	10		96	15		89	7		0.01
GC	Ethofumesate	93	10		98	7		98	5		0.01
LC	Ethofumesate	77	17		88	5		91	6		0.01
LC	Ethoxysulfuron	75	10		74	8		81	4		0.01
GC	Etoxazole	80	8		83	7		85	5		0.01
LC	Fenpyroximate	77	8		82	2		91	4		0.01
GC	Flumioxazin	72	14		83	9		88	5		0.01
LC	Forchlorfenuron	60	16		66	11		77	5		0.01
LC	Imazosulfuron	71	8		73	3		76	6		0.01

	Barley - 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**
GC	Isocarbophos	75	7		86	10		86	6		0.01
LC	Meptyldinocap	119	24		96	18		102	18		0.01
GC	Metobromuron	a, e, g			a, e, g			71	7		0.1
LC	Metobromuron	72	19		71	14		79	3		0.01
LC	Nicosulfuron	60	12		55	6		58	3		0.01
GC	Propachlor	85	17		94	15		93	8		0.01
LC	Propachlor	79	12		84	5		90	3		0.01
LC	Propoxycarbazone sodium salt	78	8		77	8		81	3		0.01
LC	Pyraflufen ethyl	79	9		83	5		93	2		0.01
GC	Pyraflufen-ethyl	83	5		90	5		91	5		0.01
LC	Rotenone	81	14		84	7		96	5		0.01
LC	Spirotetramat	75	9		81	7		90	4		0.01
GC	Tetramethrin	78	17		86	11		85	6		0.01
LC	Tetramethrin	83	8		83	3		95	6		0.01
LC	Trineexpac-ethyl	a			55	6		52	5		0.02
Pesticides/metabolites not possible to validate											
LC	Asulam	a, e									
LC	Foramsulfuron	e									
LC	TFNA	h									

a) RSDr > 20%; e) Recovery <50%; g) To low sensitivity; h) not multimetod amenable.

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

	Rice - 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	2-hydroxypropoxycarbazone	71	8		69	7		72	4		0.01
GC	Acibenzolar-S-methyl	101	9		101	2		99	4		0.01
GC	Anthraquinone	90	7		98	3		95	4		0.01
LC	Avermectin B1a	101	16		102	13		94	15		0.01
GC	Beflubutamid	89	5		98	2		105	2		0.01
GC	Bifenazate	83	5		76	6		69	2		0.01
LC	Bifenazate	63	10		66	9		61	6		0.01
GC	Biphenyl	*148*	*17*		*124*	10		99	3		0.02
GC	Butralin	104	7		98	5		95	6		0.01
LC	Butralin	78	24		87	8		102	6		0.01
LC	Carfentrazone ethyl	84	13		96	8		100	7		0.01
GC	Carfentrazone-ethyl	95	4		101	4		102	3		0.01
LC	Chlorotoluron	93	6		96	4		97	4		0.01
GC	Dichlobenil	109	10		102	4		99	4		0.01
GC	Dimethenamid	93	6		97	3		98	2		0.01
LC	Dimethenamid	95	3		93	2		92	3		0.01
GC	Ethalfluralin	99	9		104	7		105	5		0.01
GC	Ethofumesate	96	6		104	8		105	3		0.01
LC	Ethofumesate	78	14		91	6		94	2		0.01
LC	Ethoxysulfuron	65	9		67	6		75	4		0.01
GC	Etoxazole	95	9		97	6		100	3		0.01
LC	Fenpyroximate	92	5		98	4		103	4		0.01
GC	Flumioxazin	99	9		100	8		96	4		0.01
LC	Forchlorfenuron	88	13		82	6		78	5		0.01
LC	Imazosulfuron	65	17		66	5		70	6		0.01

	Rice - 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**
GC	Isocarbophos	113	10		121	7		115	3		0.01
LC	Meptyldinocap		a		95	19		103	9		0.02
GC	Metobromuron	a, e, g			a, e, g			98	14		0.1
LC	Metobromuron	100	11		87	7		86	6		0.01
LC	Nicosulfuron	53	1		50	9		53	7		0.01
GC	Propachlor	119	20		102	9		94	6		0.01
LC	Propachlor	92	5		98	4		104	4		0.01
LC	Propoxycarbazone sodium salt	78	3		81	7		82	5		0.01
LC	Pyraflufen ethyl	86	4		96	5		99	4		0.01
GC	Pyraflufen-ethyl	95	6		101	5		102	3		0.01
LC	Rotenone	90	9		97	5		102	3		0.01
LC	Spirotetramat	87	7		94	4		98	4		0.01
GC	Tetramethrin	101	15		108	9		96	5		0.01
LC	Tetramethrin	89	5		99	6		107	3		0.01
LC	Trineexpac-ethyl	66	5		54	9		55	3		0.01
Pesticides/metabolites not possible to validate											
LC	Asulam	a, e									
LC	Foramsulfuron	e									
LC	TFNA	h									

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 6: 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.