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

Validation Report 13

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

(QuEChERS method)

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December 2013

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 45 pesticides in wheat, rye, rice and barley. 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, 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 tube and put in -80 degree freezer. When the extract is almost thawed it is centrifuged and the supernatant is transferred to a tube with 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 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 detected by tandem mass spectrometry (MS/MS) operating with electron energy at 70 eV, source temperature at 180°C and transfer line at 250°C. The injection volume was 4 µl. All pesticides were detected in the multiple reaction monitoring mode (MRM). For each pesticide two transition 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 for quantification. All pesticides were detected in the multiple reaction monitoring mode (MRM). 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 45 pesticides or degradation products in wheat, rye, 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.

Pesticides included in recovery experiments		
1-Naphthylacetamide	Emamectin benzoate	Metosulam
Acetochlor	Etridiazole	Metrafenone
Ametoctradin	Fluazinam	Napropamide
Benfluralin	Fluometuron	Oryzalin
Bromadiolone	Fluopicolide	Oxadiargyl
Carvone	Fluopyram	Oxyfluorfen
Chloridazon	Flurochloridone	Penflufen
Chlorsulfuron	Flurprimidole	Penthiopyrad
Cyflumetofen	Fluxapyroxad	Pethoxamide
Cymoxanil	Halosulfuron-methyl	Phenmedipham
Dazomet	Imazamox	Pyroxsulam
Desmedipham	Imazaquin	Spiromesifen
Dimethachlor	Isopyrazam	Sulcotrione
Dodemorph	Metaldehyde	Terbuthylazine
Dodine	Metazachlor	Tritosulfuron

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 are presented in **Figure 5-8**.

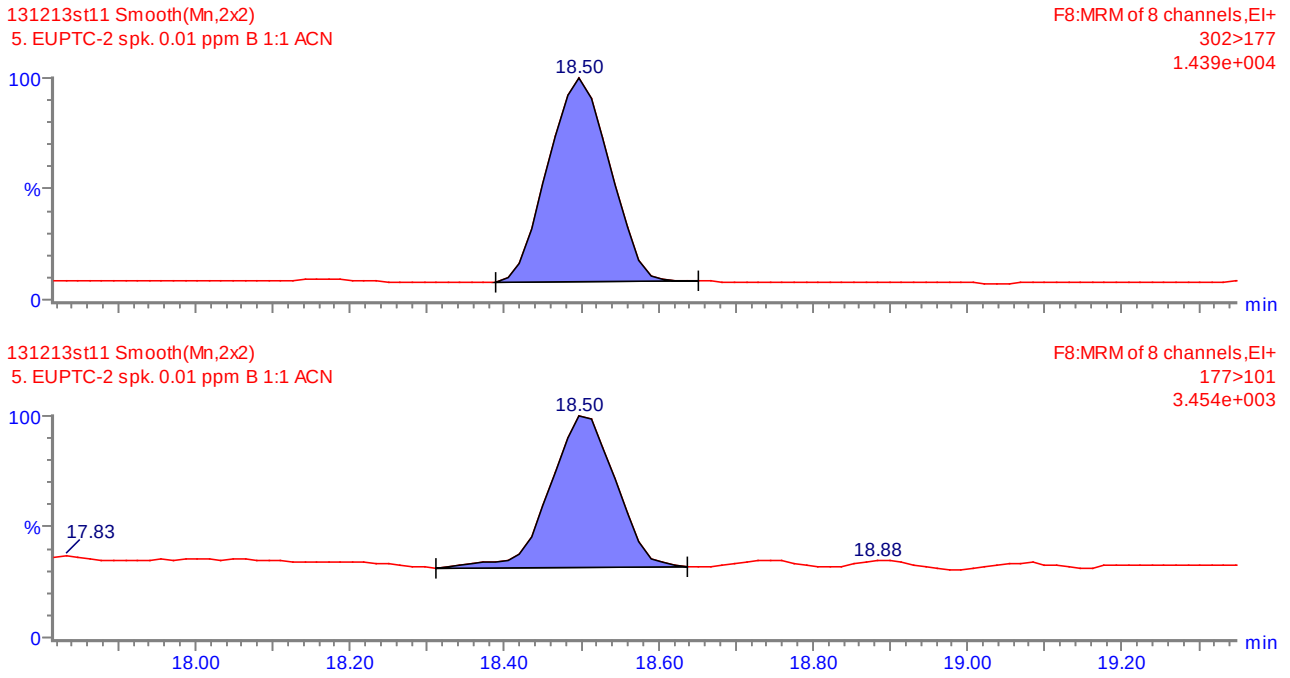

Figure 1: Examples of GC-MS/MS chromatograms for penthiopyrad/wheat and tritosulfuron/rice obtained when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown for each pesticide).

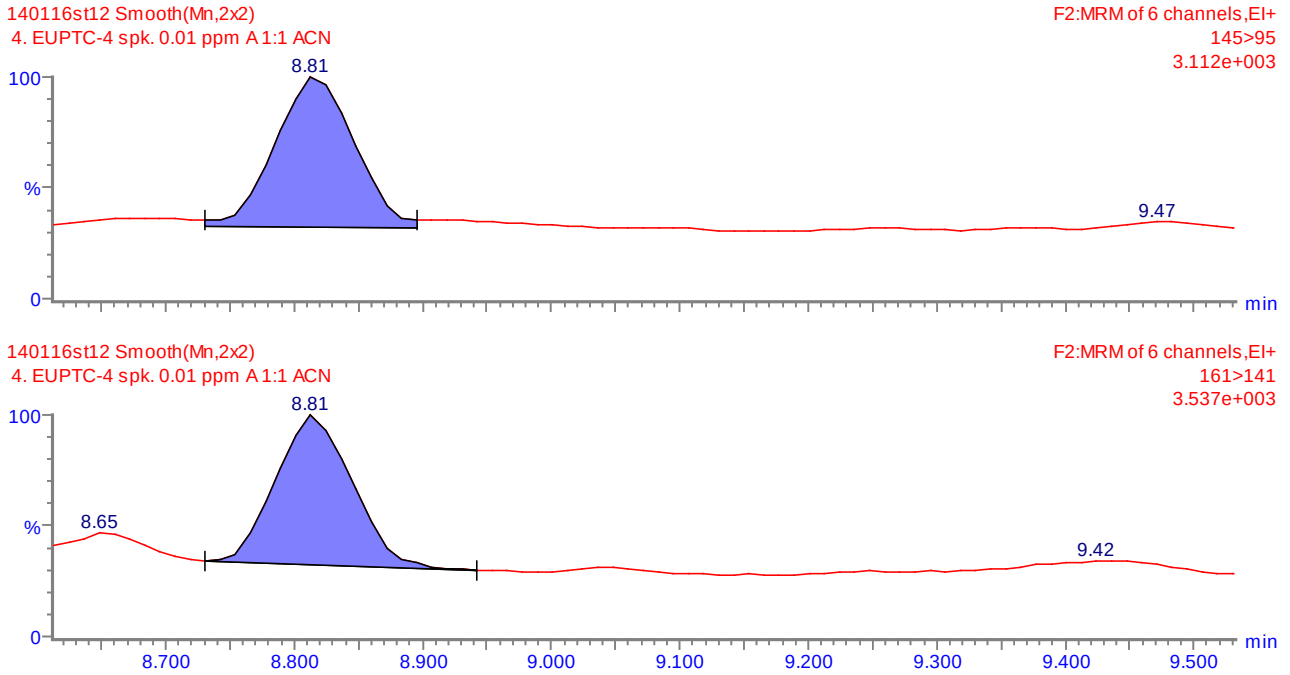

Figure 2: Examples of GC-MS/MS chromatograms tritosulfuron/rye obtained when analysing extract spiked with 0.01 mg/kg .

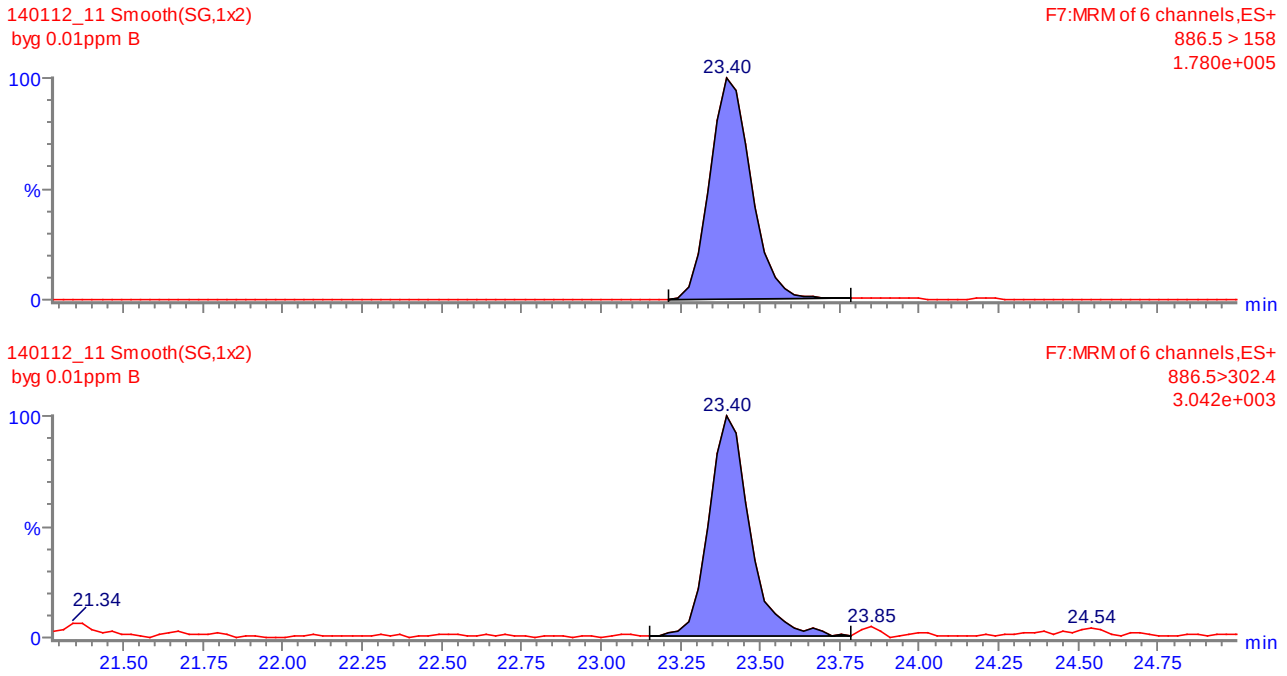

Figure 3: Examples of LC-MS/MS chromatograms emamectin benzoate/barley obtained in positive mode when analysing extract spiked with 0.01 mg/kg.

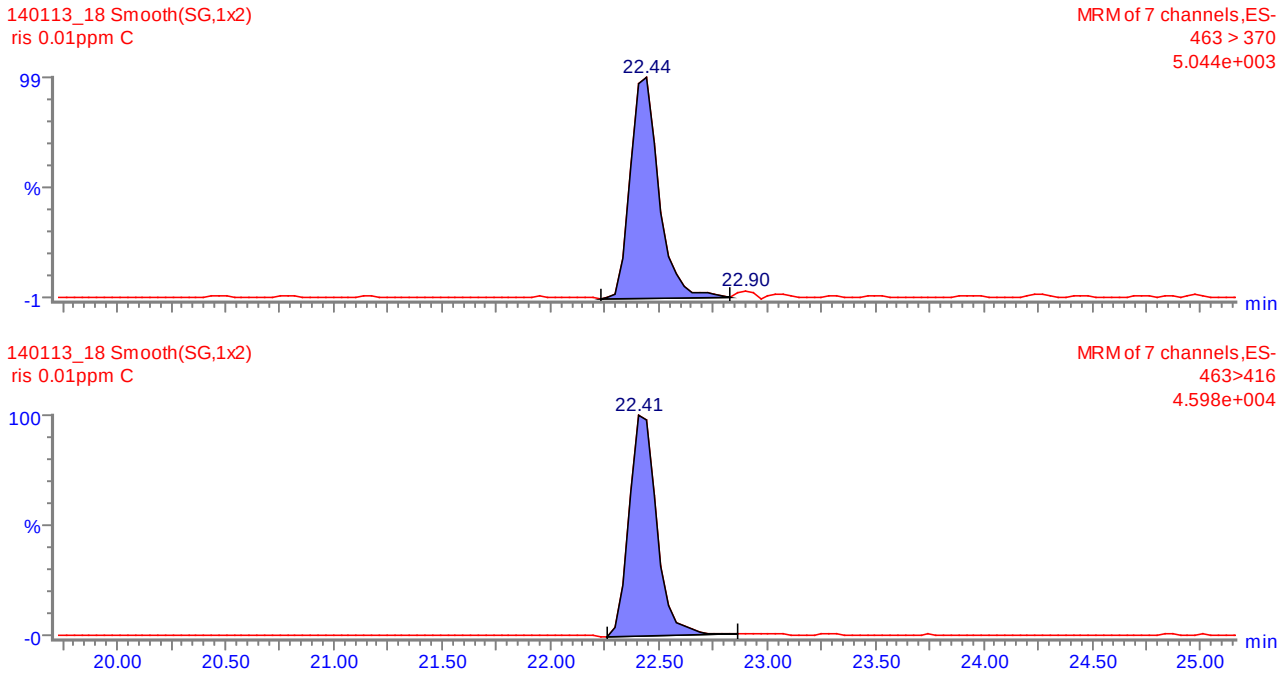

Figure 4: Examples of LC-MS/MS chromatograms fluzianam/rice obtained when analysing extract in negative mode spiked with 0.01 mg/kg

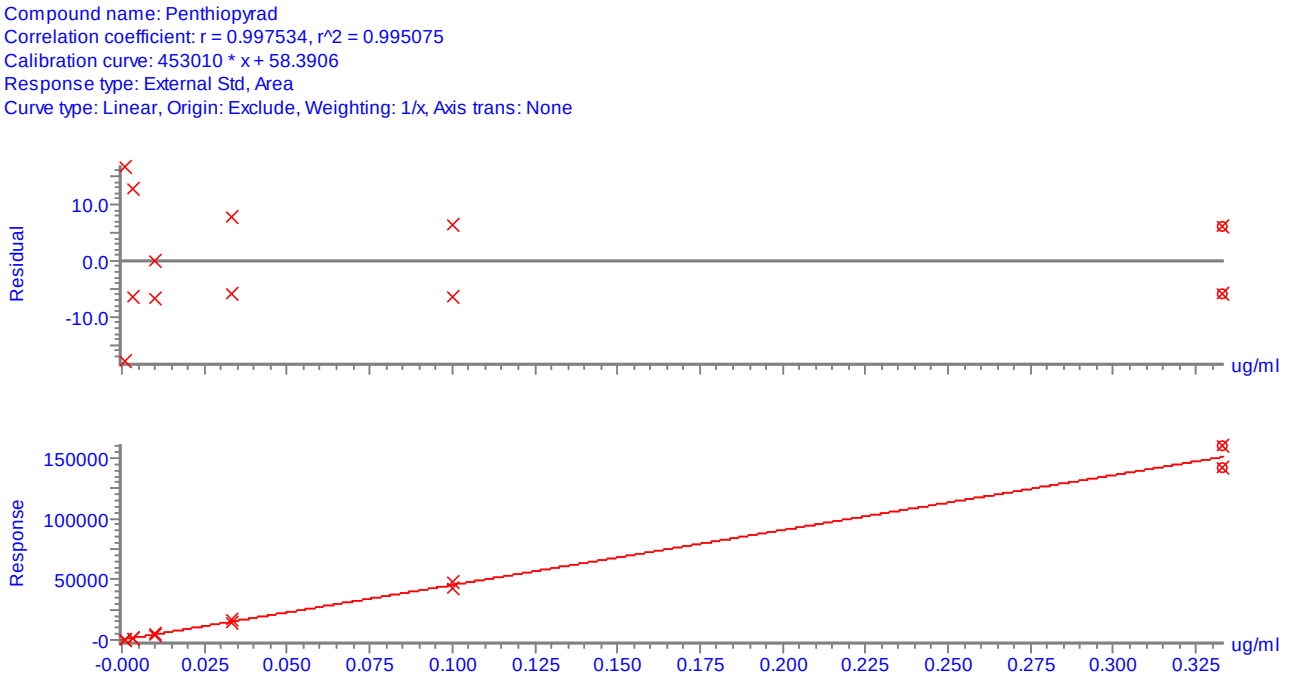

Figure 5. Examples of GC-MS/MS calibration curves for penthiopyrad (concentrations from 0.003-0.333 µg/ml)

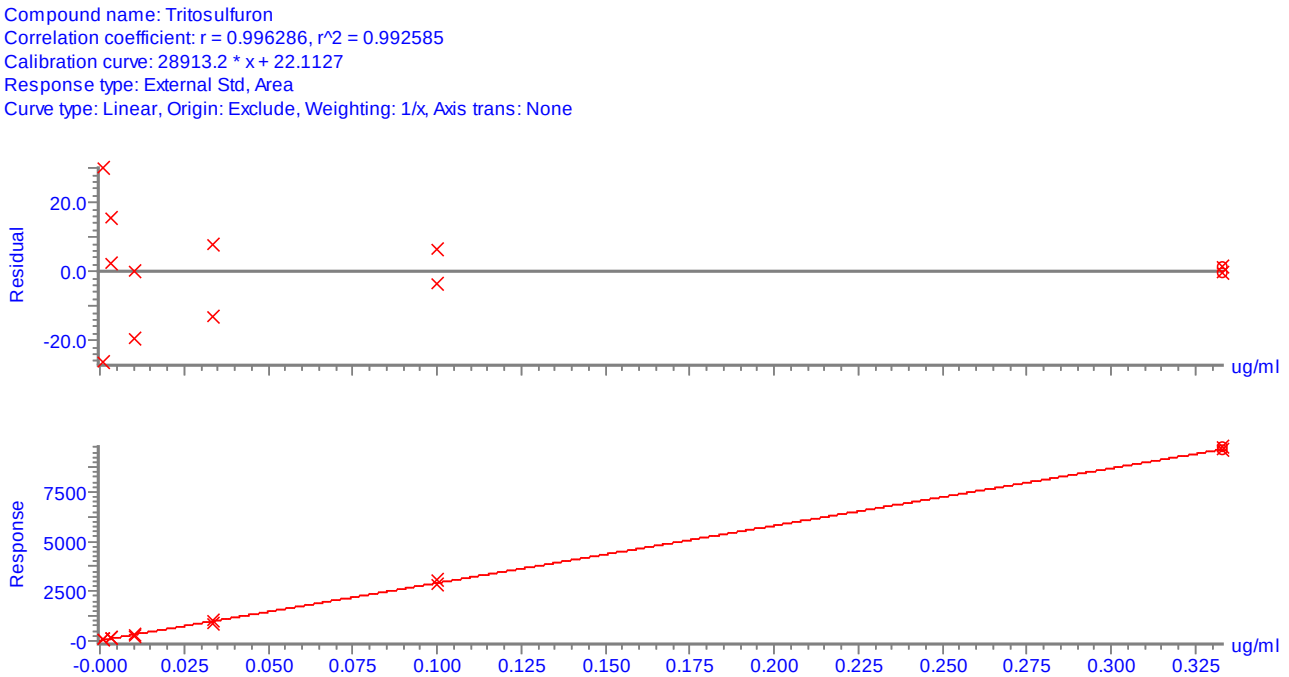

Figure 6. Examples of GC-MS/MS calibration curves for tritosulfuron concentrations from 0.003-0.333 µg/ml)

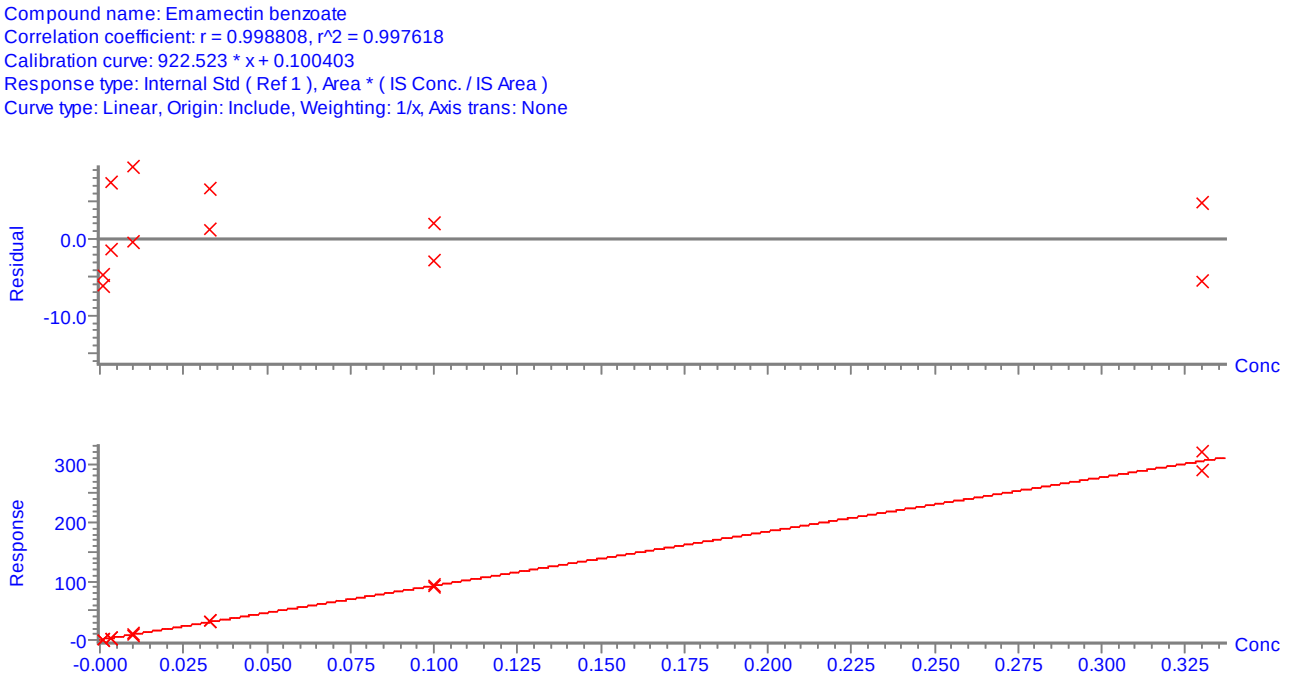

Figure 7. Examples of LC-MS/MS calibration curves for emamectin benzoate (concentrations from 0.003-0.333 µg/ml).

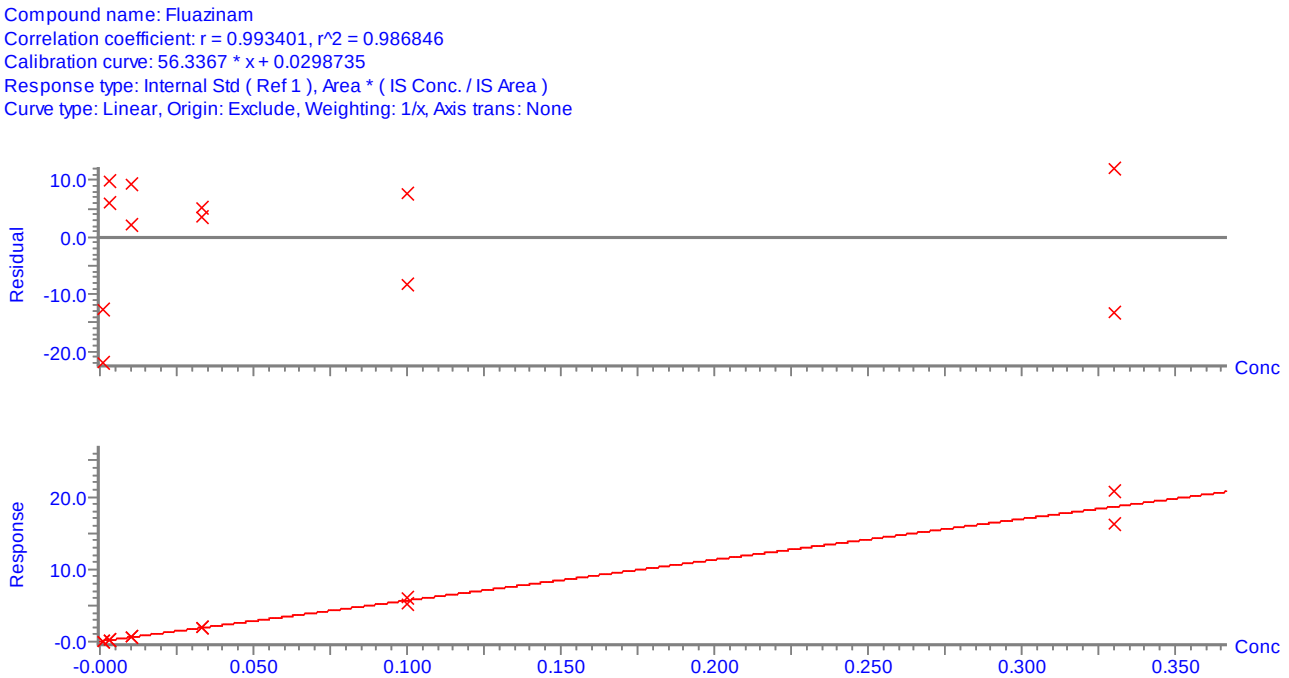

Figure 8. Examples of LC-MS/MS calibration curves for fluazinam (concentrations from 0.003-0.333 µg/ml)

5. Validation parameters

Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides and degradation products on all three spiking levels, 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 and internal reproducibility in this validation was calculated from the 5-6 replicate determinations. Repeatability were calculated as given in ISO 5725-2[2].

Appendix 2-6 shows the relative repeatability and internal reproducibility for the validated pesticides and degradation products.

Accuracy – Recovery

The accuracy was determined by recovery, samples were spiked at three concentration levels. In appendix 2 and 3 recovery, repeatability and limit of quantification (LOQ) are given for the validated pesticides, isomers and degradation products for all three spiking levels (0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg). Recoveries is listed in **Appendix 2-6**.

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**.

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

Overall validation on all 4 cereal types.

Thirty-seven pesticides were validated on all four cereal types either analysed by GC-MS/MS (7 pesticides), LC-MS/MS (10 pesticider) or both (20 pesticides), see **Appendix 2**. Dazomet, Dodine, imazamox, imazaquin, metosulam, oryzalin, pyroxsulam and sulcotrione was not validated for all cereal type together. However, oryzalin was validated on rice. Dodine, pyroxsulam and sulcotrione was all validated on rye. For the other cereal type the recoveries were too low. Validation for bromadiolone, carvone and metaldehyde was only accepted for the highest spike level.

The relative repeatability (RSD_r) varied between 3-25 % with an average on 9%. The internal reproducebility (RSD_R) varied between 3-36% with an average on 14%. Recoveries was in the range of 62-121% at all three concentration levels with an average on 99%. The combined LOQs were in the range of 0.003-0.1 mg/kg.

Validation on individual cereal type.

Wheat: Carvone was not validated and the validation for bromadiolone and metaldehyde could not be accepted at the two lowest spike level (0.01 and 0.02 mg/kg).

Rye: Bromadiolone was not validated and validation for acetochlor, cymoxanil and etridiazole could not be accepted at the lowest spike level (0.01) mg/kg and carvone and sulcotrione was not accepted for the two lowest spike level (0.01 and 0.02 mg/kg). However, dodine, pyroxsulam and sulcotrione was validated with fine recoveries and low repeatabilities in rye.

Rice: Validation for bromadiolone, carvone, chloridazon, metaldehyde and oryzalin was not accepted for the two lowest spike level (0.01 and 0.02 mg/kg). Oryzalin was validated on rice only, but only at the higest spike level (0.1 mg/kg).

Barley: Validation for cymoxanil, etridiazole and fluzianam was not be accepted at the lowest spike level (0.01) mg/kg and bromadiolone, carvone and metaldehyde was not accepted for the two lowest spike level (0.01 and 0.02 mg/kg).

The results for the pesticides analysed on GC-MS/MS and LC/MS/MS are listed in **Appendix 2-6**.

8. Conclusions

In conclusion 37 pesticides were validated on wheat, oat , rye, rice and barley for the QuEChERS method using GC-MS/MS and LC-MS/MS for the detection. For wheat and barley 36 pesticides, rye 39 pesticides and rice 38 pesticides were validated. Bromadiolone, carvone, metaldehyde and

oxadiargyl was only validated on the highest spike level (0.1 mg/kg) and further experiments should be performed.

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
1	1-Naphtylacetamide	13.44	141	115	15	185	141	15
2	Acetochlor	12.78	224	148	20	223	146	20
3	Ametoctradin	9.39	149	121	5	177	149	5
4	Benfluralin (Benefin)	10.18	292	264	10	292	160	10
5	Carvone	7.11	108	93	10	82	54	15
6	Chloridazon	19.68	220	193	23	220	166	23
7	Chlorsulfuron	10.61	175	111	5	191	127	5
8	Cyflumetofen	22.66	173	145	10	174	146	20
9	Dazomet	10.82	162	89	8			
10	Dimethachlor	12.62	197	148	10	199	148	10
11	Dodemorph-1	14.64/15.14	154	136	10	154	97	10
12	Etridiazole	8.37	213	185	10	211	183	15
13	Fluazinam	15.27	420	375	20	418	373	20
14	Fluopicolide	19.85	347	172	25	209	182	17
15	Fluopyram	15.42	173	145	5	396	223	10
16	Flurochloridone	14.5	311	187	15	311	174	15
17	Flurprimidol	12.6	269	107	15	189	161	10
18	Halosulfuron methyl	12.73	224	148	5	255	197	5
19	Imazaquin	19.05	155	128	10	224	181	10
20	Isopyrazam	24.08	359	303	5	303	262	10
21	Metaldehyde	5.9	89	45	10	117	45	10
22	Metrafenone	24	393	363	20	393	335	20
23	Napropamide	16.66	128	72	10	271	128	5
24	Oryzalin	24.55	317	275	15	275	194	15
25	Oxadiargyl	18.35	340	150	15	213	150	5
26	Oxyfluorfen	17.35	274	239	15	300	223	10
27	Penflufen	18.93	274	141	10	141	84	10
28	Penthiopyrad	18.55	302	177	15	177	101	15

GC-MS/MS		Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CE
29	Pethoxamide	15.6	260	147	15	131	91	10
30	Spiromesifen	20.95	272	254	10	272	209	10
31	Sulcotrione	24.69	290	211	15	211	155	10
32	Terbuthylazine	11.34	229	173	10	229	214	10
33	Tritosulfuron	8.8	145	95	5	161	141	5

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
1	1-Naphtylacetamide	10.87	185.8	141	25	25	186.1	115.1	25	40
2	Acetochlor	18.34	270	148	25	25		133	25	25
3	Ametoctradin	21.81	277	149.9	45	35		176.9	45	35
4	Bromadiolone*	21.34	525.9	251	50	40		93	50	25
5	Chloridazon	9.72	222	104	50	25		92.1	50	25
6	Chlorsulfuron	12.00	357.9	167	50	25		141	50	25
7	Cymoxanil	10.05	199.1	111	34	15		128.2	34	15
8	Desmedipham	15.29	318,1	181.8	25	15		136	25	15
9	Dimethachlor	15.09	257.8	226	50	25		148	50	40
10	Dodemorph	16.15	282	116	25	25		98	25	35
11	Dodine	21.55	228	57.3	45	35		60.3	45	25
12	Emamectin benzoate	23.34	886.5	158	45	35		302.4	45	35
13	Fluazinam*	22.48	463	416	50	20		370	50	40
14	Fluometuron	13.92	233.1	72.2	50	20		160	50	25
15	Fluopicolide	17.00	383	173	20	20	385	175	20	20
16	Fluopyram	17.70	397	172.9	45	25		145	45	45
17	Flurochloridone	17.73	312	292	50	25		212	50	25
18	Flurprimidol	17.22	313	269.9	45	25		184.9	45	35
19	Fluxapyroxad	17.21	382	342	25	25		362	24	15

LC-MS/MS		Retention time	Precursor ion-1	Product ion-1	CV	CE	Precursor ion-2	Product ion-2	CV	CE
20	Halosulfuron-methyl	16.38	435	182	45	25		138.9	45	25
21	Imazamox	8.80	306	261	45	22		246	45	22
22	Imazaquin	11.15	312.1	198.9	50	25		266.9	50	20
23	Isopyrazam	21.17	360.3	243.9	45	20		320	45	20
24	Metazachlor	13.08	278	134.1	30	30		210.2	30	20
25	Metosulam	12.48	417.9	174.9	25	45		140	25	20
26	Metrafenone	20.81	410	209.9	45	15		226.8	45	15
27	Napropamide	18.38	272.9	129	50	20		171.9	50	20
28	Oxadiargyl	20.28	341	223	50	20		258	50	20
29	Oxyfluorfen	22.17	362	316	50	15		237	50	25
30	Penflufen	19.38	318	233.9	45	15		141	45	20
31	Penthiopyrad	19.54	360	275.9	25	15		255.9	25	5
32	Pethoxamide	18.09	296.2	131	25	25		116	25	40
33	Phenmedipham	16.65	318	136	50	25		167.8	50	20
34	Pyroxsulam	11.99	435	194.9	25	35		257.8	25	35
35	Spiromesifen	23.35	371	273	25	15		255	25	20
36	Sulcotrione	9.47	329	139	50	20		69.2	50	40
37	Terbuthylazine	16.94	231.9	176	50	20		134	50	25
38	Tritosulfuron*	13.60	444.4	192.9	40	25		135.9	40	35

* negative mode

Appendix 2. Recoveries, repeatability (RSD_r), internal reproducibility (RSDR) and Limit of Quantification (LOQ) for pesticides validated on 5 cereal commodities, wheat, oat, rye, rice and barley.

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

	Wheat, oat, rye, rice and barley - QuEChERS	Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.02	29		0.1	23		
		Recovery, %	**RSDr, %**	**RSDR, %**	**Recovery, %**	**RSDr, %**	**RSDR, %**	**Recovery, %**	**RSDr, %**	**RSDR, %**	**LOQ**
LC	1-Naphthylacetamide	99	9	13	104	6	9	101	4	7	0.007
GC	1-Naphthylacetamide	90	8	14	88	8	12	87	6	16	0.007
GC	Acetochlor	100	14	24	95	15	15	86	10	13	0.01
LC	Ametoctradin	84	8	13	100	6	8	109	5	12	0.006
GC	Benfluralin	110	18	*28*	98	12	14	91	10	14	0.02
LC	Bromadiolone							95	17	*22*	0.1
GC	Carvone							80	18	*23*	0.10
GC	Chloridazon							78	20	*26*	0.1
LC	Chloridazon	96	7	11	95	6	7	95	4	7	0.01
GC	Chlorsulfuron	*64*	20	*35*	*62*	*22*	*25*	*65*	10	19	0.01
LC	Chlorsulfuron	76	11	*26*	79	9	13	79	7	*21*	0.01
GC	Cyflumetofen	101	11	*25*	99	11	*22*	91	8	*28*	0.01
LC	Cymoxanil	108	*25*	*27*	101	14	14	101	5	7	0.03
LC	Desmedipham	115	4	6	108	6	8	101	4	10	0.004
GC	Dimethachlor	105	10	16	97	9	9	91	7	13	0.01
LC	Dimethachlor	107	9	14	110	6	12	109	5	9	0.008
LC	Dodemorph	96	5	9	93	5	9	94	4	9	0.005
GC	Dodemorph	101	12	*23*	98	12	18	95	9	*23*	0.01
LC	Emamectin benzoate	103	6	10	104	5	8	108	4	13	0.01
GC	Etridiazole	103	19	*36*	90	17	17	83	13	25	0.02
LC	Fluazinam	89	16	*23*	92	13	17	107	9	15	0.01
LC	Fluometuron	104	5	8	103	6	8	103	4	10	0.004
GC	Fluopicolide	105	8	15	103	7	16	99	6	19	0.01
LC	Fluopicolide	103	7	9	100	9	11	97	6	13	0.005
GC	Fluopyram	105	9	14	102	9	15	98	7	16	0.01

	Wheat, oat, rye, rice and barley - QuEChERS	Spike level mg/kg	Horwitz, %			Spike level mg/kg	Horwitz, %			Spike level mg/kg	Horwitz, %		
		0.01	32			0.02	29			0.1	23		
		Recovery, %	**RSDr, %**	**RSDR, %**		**Recovery, %**	**RSDr, %**	**RSDR, %**		**Recovery, %**	**RSDr, %**	**RSDR, %**	**LOQ**
LC	Fluopyram	104	5	7		103	5	6		103	4	9	0.004
GC	Flurochloridone	106	10	11		106	9	20		99	9	20	0.01
LC	Flurochloridone	103	12	16		111	10	15		112	6	13	0.01
GC	Flurprimidol	105	9	16		96	7	9		91	7	12	0.01
LC	Flurprimidol	101	8	15		109	6	12		111	4	12	0.008
LC	Fluxapyroxad	104	4	6		103	5	6		102	4	10	0.003
GC	Halosulfuron-methyl	*121*	*23*	*24*		95	*22*	*22*		87	11	15	0.02
LC	Halosulfuron-methyl	103	6	11		99	5	9		98	5	12	0.01
GC	Isopyrazam	98	11	11		101	9	20		97	7	22	0.007
LC	Isopyrazam	103	7	12		109	6	12		110	4	12	0.01
GC	Metaldehyde									77	*21*	*26*	0.1
LC	Metazachlor	104	5	8		104	6	8		103	4	10	0.00
GC	Metrafenone	97	16	17		99	12	16		96	8	20	0.009
LC	Metrafenone	102	6	10		102	7	8		100	5	10	0.01
GC	Napropamide	104	7	11		104	7	17		100	6	15	0.007
LC	Napropamide	101	8	10		106	7	9		105	6	11	0.01
GC	Oxadiargyl	101	16	16		103	15	17		104	8	18	0.01
LC	Oxadiargyl									105	10	15	0.09
GC	Oxyfluorfen	97	11	11		98	8	17		94	6	19	0.007
LC	Oxyfluorfen	96	*23*	*21*		101	14	17		97	8	11	0.02
GC	Penflufen	106	7	11		102	7	13		99	6	17	0.007
LC	Penflufen	103	7	12		112	5	10		114	3	11	0.01
GC	Penthiopyrad	109	7	13		108	8	19		104	6	22	0.008
LC	Penthiopyrad	105	7	14		111	6	12		112	4	11	0.01
GC	Pethoxamide	109	12	14		99	11	10		95	8	12	0.009
LC	Pethoxamide	102	6	11		111	5	10		112	3	9	0.01
LC	Phenmedipham	109	6	11		112	6	11		111	4	8	0.007
LC	Spiromesifen	87	12	14		98	10	11		108	6	10	0.01
GC	Spiromesifin	99	8	10		99	11	14		95	7	13	0.006

	Wheat, oat, rye, rice and barley - QuEChERS	Spike level mg/kg	Horwitz, %			Spike level mg/kg	Horwitz, %			Spike level mg/kg	Horwitz, %			
		0.01	32			0.02	29			0.1	23			
		Recovery, %	**RSDr, %**	**RSDR, %**		**Recovery, %**	**RSDr, %**	**RSDR, %**		**Recovery, %**	**RSDr, %**	**RSDR, %**		**LOQ**
GC	Terbuthylazine	106	12	*23*		96	9	11		88	8	12		0.01
LC	Terbuthylazine	102	6	14		106	5	11		104	4	10		0.008
GC	Tritosulfuron	89	*21*	*27*		86	17	17		87	9	18		0.01

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

	Wheat - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
GC	1-Naphthylacetamide	75	7		74	*22*		85	6		0.003
LC	1-Naphthylacetamide	107	6		110	8		100	5		0.004
GC	Acetochlor	99	9		90	*25*		96	6		0.01
LC	Ametoctradin	73	12		93	3		106	5		0.005
GC	Benfluralin	99	7		88	*22*		98	8		0.004
LC	Bromadiolone							78	15		0.02
GC	Chloridazon							72	13		0.06
LC	Chloridazon	84	5		90	6		92	4		0.003
LC	Chlorsulfuron	69	3		77	7		68	10		0.001
GC	Chlorsulfuron	85	22		*69*	*25*		66	6		0.01
GC	Cyflumetofen	*131*	5		116	*24*		*122*	4		0.004
LC	Cymoxanil	109	18		102	19		101	3		0.02
LC	Desmedipham	111	3		100	4		98	4		0.002
GC	Dimethachlor	98	4		90	*22*		102	4		0.003
LC	Dimethachlor	109	9		*122*	7		111	5		0.006
LC	Dodemorph	85	2		81	2		88	4		0.001
GC	Dodemorph	71	15		79	20		87	8		0.01
LC	Emamectin benzoate	91	5		94	3		101	4		0.003
GC	Etridiazole	97	13		89	*25*		108	8		0.01
GC	Fluazinam							95	19		0.1
LC	Fluazinam	71	12		80	15		94	8		0.005
LC	Fluometuron	96	3		95	2		99	4		0.002
GC	Fluopicolide	99	5		87	*22*		100	4		0.003
LC	Fluopicolide	97	4		94	4		96	6		0.002
GC	Fluopyram	99	6		87	18		95	5		0.004

	Wheat - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	Fluopyram	95	4		97	4		99	4		0.002
GC	Flurochloridone	103	7		87	19		99	6		0.004
LC	Flurochloridone	101	6		*121*	10		114	7		0.004
GC	Flurprimidol	99	5		86	*22*		96	4		0.003
LC	Flurprimidol	101	3		112	7		105	5		0.002
LC	Fluxapyroxad	99	3		96	3		99	4		0.002
GC	Halosulfuron-methyl	108	19		93	16		96	7		0.01
LC	Halosulfuron-methyl	97	7		94	2		92	5		0.004
GC	Isopyrazam	91	5		80	21		90	4		0.002
LC	Isopyrazam	101	2		109	8		104	3		0.001
GC	Metaldehyde							69	13		0.05
LC	Metazachlor	94	4		96	3		98	4		0.003
GC	Metrafenone	105	8		86	*24*		97	5		0.01
LC	Metrafenone	93	4		95	4		97	4		0.002
GC	Napropamide	101	4		90	23		98	3		0.002
LC	Napropamide	93	8		96	7		100	4		0.004
GC	Oxadiargyl	103	12		95	*23*		106	6		0.01
LC	Oxadiargyl							103	12		0.07
GC	Oxyfluorfen	92	11		87	18		98	2		0.01
LC	Oxyfluorfen	99	18		86	*22*		95	9		0.01
GC	Penflufen	97	5		87	*24*		98	3		0.003
LC	Penflufen	103	3		114	5		106	3		0.002
GC	Penthiopyrad	101	6		89	22		100	5		0.004
LC	Penthiopyrad	102	2		110	6		105	3		0.001
GC	Pethoxamide	105	5		92	*21*		100	3		0.003
LC	Pethoxamide	99	3		108	6		107	2		0.002
LC	Phenmedipham	109	4		115	7		108	4		0.002
LC	Spiromesifen	91	*21*		99	15		109	5		0.02

	Wheat - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
GC	Spiromesifen	108	7		92	21		106	4		0.004
GC	Terbuthylazine	90	5		83	*23*		95	3		0.003
LC	Terbuthylazine	101	3		107	7		99	4		0.002
GC	Tritosulfuron	113	19		93	*22*		101	7		0.01

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

	Rye - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
GC	1-Naphthylacetamide	95	13		97	10		100	3		0.007
LC	1-Naphthylacetamide	107	12		110	7		109	3		0.008
GC	Acetochlor				98	23		84	4		0.03
LC	Ametoctradin	80	5		98	6		119	5		0.003
GC	Benfluralin				111	15		101	9		0.02
GC	Carvone							99	17		0.1
GC	Chloridazon							97	*23*		0.1
LC	Chloridazon	102	6		100	10		100	4		0.004
LC	Chlorsulfuron	103	16		90	10		102	7		0.01
GC	Chlorsulfuron							78	9		0.04
GC	Cyflumetofen	77	20		84	10		*64*	13		0.009
LC	Cymoxanil				93	14		102	3		0.02
LC	Desmedipham	121	3		115	8		112	4		0.002
GC	Dimethachlor				99	12		89	2		0.02
LC	Dimethachlor	123	10		*117*	8		120	3		0.007
LC	Dodemorph	99	5		93	7		101	4		0.003
GC	Dodemorph	118	12		116	13		*124*	5		0.01
LC	Dodine	82	*24*		79	13		113	14		0.01
LC	Emamectin benzoate	113	5		110	8		121	3		0.004
GC	Etridiazole				*93*	11		74	7		0.01
LC	Fluazinam	102	*23*		102	17		120	11		0.01
LC	Fluometuron	111	5		110	7		113	5		0.003
GC	Fluopicolide	125	11		*125*	9		123	5		0.008
LC	Fluopicolide	111	4		111	10		110	4		0.003
GC	Fluopyram	122	11		120	12		118	5		0.008

	Rye - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	Fluopyram	109	2		107	5		110	3		0.001
GC	Flurochloridone	116	12		*128*	7		122	9		0.009
LC	Flurochloridone	119	15		*123*	10		128	5		0.01
GC	Flurprimidol				104	11		98	3		0.01
LC	Flurprimidol	119	11		*123*	9		*126*	3		0.008
LC	Fluxapyroxad	107	4		106	7		110	4		0.002
GC	Halosulfuron-methyl							85	15		0.08
LC	Halosulfuron-methyl	117	5		105	8		112	4		0.004
GC	Isopyrazam	98	19		*127*	9		*126*	4		0.01
LC	Isopyrazam	119	9		*124*	7		*127*	3		0.007
LC	Metazachlor	109	*2*		110	*8*		113	3		0.00
LC	Metosulam	111	*23*		85	15		104	10		0.02
GC	Metrafenone	95	*22*		115	13		120	8		0.01
LC	Metrafenone	99	5		104	11		109	5		0.003
GC	Napropamide	118	9		*127*	8		117	3		0.006
LC	Napropamide	103	12		110	10		117	4		0.007
GC	Oxadiargyl	93	12		117	10		*124*	7		0.01
LC	Oxadiargyl							115	11		0.08
GC	Oxyfluorfen	108	8		118	10		115	3		0.01
LC	Oxyfluorfen				110	13		108	6		0.02
GC	Penflufen	119	7		120	9		119	3		0.005
LC	Penflufen	117	10		*125*	8		128	2		0.007
GC	Penthiopyrad	126	7		*135*	11		*135*	6		0.005
LC	Penthiopyrad	125	9		*127*	9		*129*	4		0.007
GC	Pethoxamide	118	13		101	14		103	4		0.009
LC	Pethoxamide	117	8		*124*	7		*125*	2		0.006
LC	Phenmedipham	123	6		*125*	8		*122*	3		0.004
LC	Pyroxsulam	95	13		*67*	17		87	10		0.01

	Rye - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	Spiromesifen	93	14		103	10		117	7		0.008
GC	Spiromesifen	97	11		110	18		99	7		0.007
LC	Sulcotrione							78	15		0.07
GC	Terbuthylazine				106	10		92	4		0.01
LC	Terbuthylazine	121	9		121	6		118	4		0.007
GC	Tritosulfuron	73	*33*		85	18		97	7		0.01

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

	Rice - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
GC	1-Naphthylacetamide	94	6		92	3		92	3		0.003
LC	1-Naphthylacetamide	93	9		103	5		101	5		0.005
GC	Acetochlor	88	15		97	10		90	9		0.01
LC	Ametoctradin	80	5		98	6		119	5		0.003
GC	Benfluralin	105	14		95	8		86	5		0.009
LC	Bromadiolone							107	14		0.09
GC	Carvone							73	15		0.07
GC	Chloridazon							82	14		0.070
LC	Chloridazon	99	9		93	4		87	6		0.006
LC	Chlorsuforon	65	7		75	6		77	3		0.003
GC	Chlorsulfuron							*63*	7		0.03
GC	Cyflumetofen	104	3		102	3		95	4		0.002
LC	Cymoxanil	95	*25*		98	12		95	8		0.01
LC	Desmedipham	115	7		106	7		90	5		0.005
GC	Dimethachlor	99	8		*99*	3		93	2		0.005
LC	Dimethachlor	95	9		99	6		100	7		0.005
LC	Dodemorph	99	6		97	5		89	6		0.004
GC	Dodemorph	109	11		101	4		92	5		0.007
LC	Emamectin benzoate	103	7		103	6		93	6		0.004
GC	Etridiazole	108	19		86	*26*		68	13		0.01
LC	Fluazinam	93	4		101	7		116	8		0.002
LC	Fluometuron	101	5		99	7		93	5		0.003
GC	Fluopicolide	95	4		101	4		94	2		0.002
LC	Fluopicolide	98	12		94	11		83	11		0.007
GC	Fluopyram	101	5		98	6		97	1		0.003

	Rice - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	Fluopyram	105	7		101	6		92	6		0.004
GC	Flurochloridone	100	9		103	6		95	4		0.005
LC	Flurochloridone	89	16		100	16		109	7		0.008
GC	Flurprimidol	99	3		98	4		92	2		0.002
LC	Flurprimidol	94	6		108	5		117	4		0.004
LC	Fluxapyroxad	101	7		102	5		91	6		0.004
GC	Halosulfuron-methyl	107	18		107	7		92	8		0.01
LC	Halosulfuron-methyl	97	7		92	5		86	5		0.004
GC	Isopyrazam	104	6		98	5		*94*	2		0.004
LC	Isopyrazam	97	6		105	5		111	5		0.004
GC	Metaldehyde							77	*24*		0.1
LC	Metazachlor	105	7		104	6		92	7		0.004
GC	Metrafenone	86	12		96	12		92	3		0.01
LC	Metrafenone	112	5		104	6		88	7		0.003
GC	Napropamide	100	7		97	5		100	2		0.004
LC	Napropamide	98	6		105	7		94	9		0.003
GC	Oryzalin							79	20		0.09
GC	Oxadiargyl	101	16		100	13		100	5		0.01
LC	Oxadiargyl							89	10		0.05
GC	Oxyfluorfen	100	7		95	6		90	3		0.004
LC	Oxyfluorfen	89	*30*		97	9		89	11		0.02
GC	Penflufen	104	3		99	5		97	2		0.002
LC	Penflufen	96	5		109	3		119	4		0.003
GC	Penthiopyrad	103	4		102	1		99	1		0.002
LC	Penthiopyrad	97	6		107	5		112	4		0.003
GC	Pethoxamide	98	11		101	6		94	4		0.006
LC	Pethoxamide	97	5		107	4		113	5		0.003
LC	Phenmedipham	99	8		104	4		113	6		0.005

	Rice - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
LC	Spiromesifen	79	10		89	12		95	6		0.005
GC	Spiromesifen	98	3		98	4		94	4		0.002
GC	Terbuthylazine	88	10		93	8		89	3		0.005
LC	Terbuthylazine	93	6		102	3		102	5		0.004
GC	Tritosulfuron	83	16		82	14		78	5		0.008

Appendix 6. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQs) for pesticides validated on barley.

	Barley - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32	Spike level, mg/kg 0.02	Horwitz, % 29	Spike level, mg/kg 0.1	Horwitz, % 23	
		Recovery %	**RSDr %**	**Recovery %**	**RSDr %**	**Recovery %**	**RSDr %**	**LOQ**
GC	1-Naphthylacetamide	96	5	81	11	69	10	0.003
LC	1-Naphthylacetamide	89	8	94	1	94	3	0.004
GC	Acetochlor	85	12	85	14	76	17	0.01
LC	Ametoctradin	92	6	104	5	118	3	0.003
GC	Benfluralin	93	10	90	14	81	17	0.005
LC	Bromadiolone					100	20	0.1
GC	Carvone					85	16	0.08
LC	Chloridazon	100	6	96	4	99	2	0.003
LC	Chlorsulfuron	67	7	72	6	*69*	8	0.003
GC	Chlorsulfuron	72	14	64	17	52	19	0.01
GC	Cyflumetofen	93	16	86	13	82	14	0.009
LC	Cymoxanil			108	10	108	4	0.01
LC	Desmedipham	111	4	107	3	104	4	0.003
GC	Dimethachlor	97	10	93	11	79	16	0.006
LC	Dimethachlor	102	7	102	3	104	5	0.004
LC	Dodemorph	101	5	99	4	101	3	0.003
GC	Dodemorph	103	12	91	7	78	18	0.01
LC	Emamectin benzoate	105	5	109	5	117	4	0.003
GC	Etridiazole			84	21	82	20	0.02
LC	Fluazinam			84	*12*	97	7	0.01
LC	Fluometuron	107	6	105	6	107	2	0.004
GC	Fluopicolide	100	8	93	11	79	12	0.005
LC	Fluopicolide	103	5	99	8	99	4	0.003
GC	Fluopyram	96	10	95	8	82	13	0.006
LC	Fluopyram	105	5	107	4	109	3	0.003

	Barley - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32	Spike level, mg/kg 0.02	Horwitz, % 29	Spike level, mg/kg 0.1	Horwitz, % 23	
		Recovery %	**RSDr %**	**Recovery %**	**RSDr %**	**Recovery %**	**RSDr %**	**LOQ**
GC	Flurochloridone	105	10	94	8	80	14	0.006
LC	Flurochloridone	101	7	100	6	95	5	0.004
GC	Flurprimidol	95	8	89	7	78	15	0.005
LC	Flurprimidol	90	6	94	2	96	4	0.003
LC	Fluxapyroxad	107	3	108	4	110	3	0.002
GC	Halosulfuron-methyl					74	14	0.06
LC	Halosulfuron-methyl	102	4	105	4	103	5	0.00
GC	Isopyrazam	99	8	90	12	77	15	0.005
LC	Isopyrazam	95	7	97	3	*98*	3	0.004
GC	Metaldehyde					*94*	21	0.1
LC	Metazachlor	107	*6*	106	4	107	2	0.004
GC	Metrafenone	101	17	90	15	78	14	0.010
LC	Metrafenone	105	10	104	3	105	3	0.01
GC	Napropamide	98	8	93	11	84	14	0.005
LC	Napropamide	108	6	110	7	110	6	0.004
GC	Oxadiargyl	106	22	98	13	84	14	0.014
LC	Oxadiargyl					114	8	0.06
GC	Oxyfluorfen	*99*	14	85	9	74	15	0.01
LC	Oxyfluorfen	99	19	110	8	96	5	0.01
GC	Penflufen	102	10	94	8	82	13	0.006
LC	Penflufen	95	5	101	2	103	3	0.003
GC	Penthiopyrad	106	11	96	8	83	12	0.007
LC	Penthiopyrad	96	6	100	2	102	3	0.004
GC	Pethoxamide	116	15	93	14	84	17	0.01
LC	Pethoxamide	96	5	103	2	104	3	0.003
LC	Phenmedipham	103	6	103	3	102	2	0.004
LC	Spiromesifen	87	11	102	4	112	4	0.006
GC	Spiromesifen	93	10	88	11	80	12	0.01

	Barley - QuEChERS	Spike level, mg/kg 0.01	Horwitz, % 32		Spike level, mg/kg 0.02	Horwitz, % 29		Spike level, mg/kg 0.1	Horwitz, % 23		
		Recovery %	**RSDr %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
GC	Terbuthylazine	100	10		94	10		77	18		0.006
LC	Terbuthylazine	93	3		95	1		96	3		0.002
GC	Tritosulfuron	85	15		84	10		73	15		0.008

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.