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Appendix 3

Validation Report 15

Determination of pesticide residues in wheat, rye, rice and barley by LC-MS/MS (SweEt method)

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December 2013

CONTENT

1. Introduction

This report describes the validation of the SweEt method combined with LC-MS/MS on barley, rice, rye and wheat. The method is a Swedish developed method based on simple and efficient process with ethyl acetate as extraction solvent[1].
This validation does not include testing of the methods robustness.

2. Principle of analysis

Sample preparation: The samples are milled with a sieve at 1.0 mm.
Extraction: The sample was mix with water and extracted with ethyl acetate with 1 % acetic acid by shaken. The sample was briefly shaken with sodium sulphate before the extraction continued by ultrasonic. The ethyl acetate and water phase are separated by centrifugation and the supernatant filtered. The final extract was diluted 1:1 with ethyl acetate to obtain the same matrix concentration as in the calibration standards.
Clean-up: The method does not include any clean-up step.
Quantification and qualification: 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 only by positive ESI. For quantification $^{13}C_6$-carbaryl was used as internal standard. 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 used for quantification and one for qualification. The MRM transitions for the pesticides and degradation products indented validated are given in **Appendix 1**.

3. Validation design

The method was south validated for 60 pesticides or degradation products in barley, rice, rye and wheat. The validation was performed on 5-6 replicates at each of the three spiking levels; 0.01, 0.02 and 0.1 mg/kg. The tests were done on different days with six replicates by two different laboratory technicians. A blank sample of each cereal commodity was included.

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at least five calibration levels, i.e. 0.003, 0.01, 0.033, 0.1, 0.333 µg/ml. The calibration curves were best fitted to a quadratic or 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 and calibration curves obtained when analysing the extracts by LC-MS/MS are presented in **figure 1 and 2**.

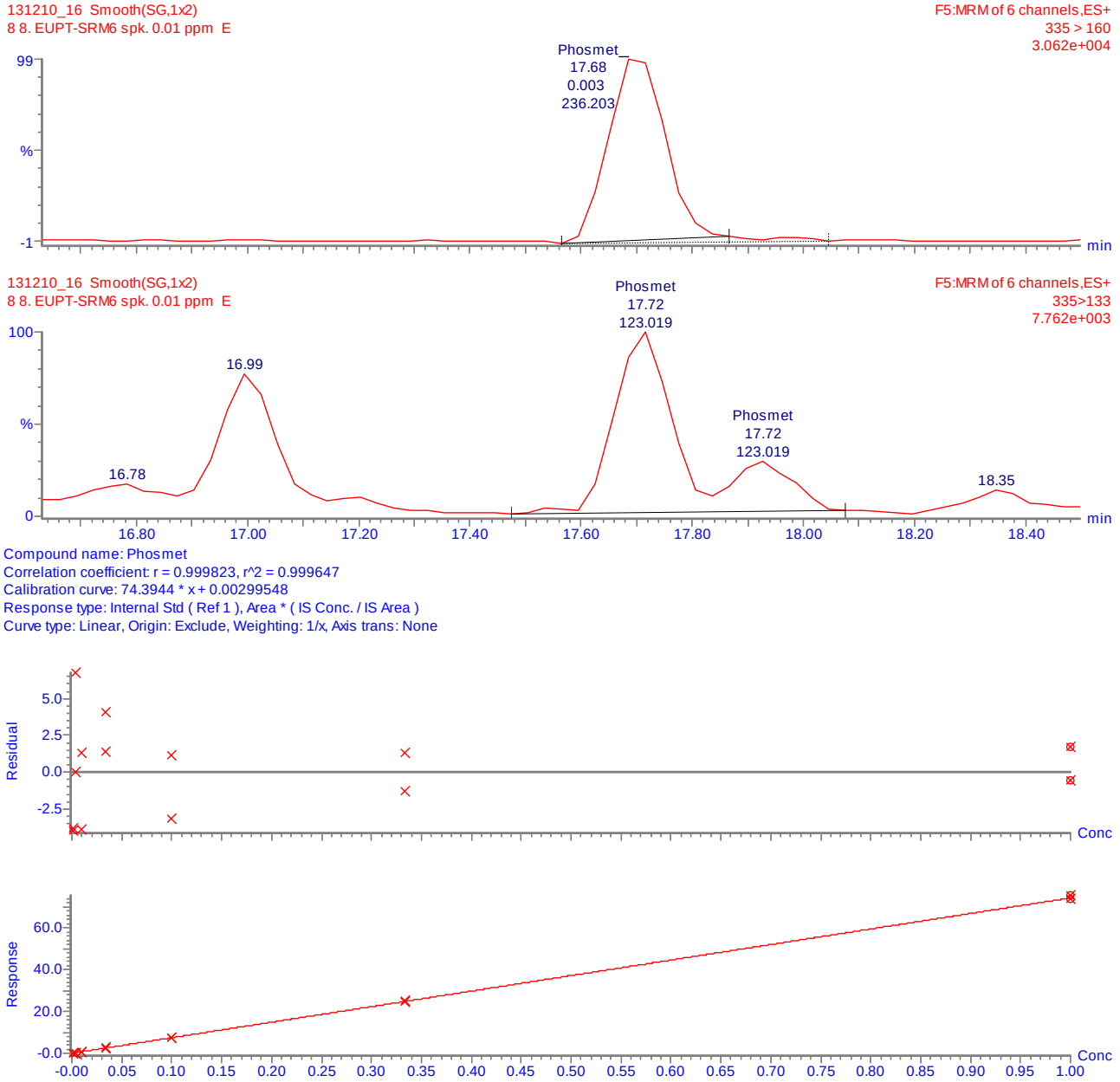

Figure 1. Examples of chromatograms, calibration curves and residual plot for phosmet in rice obtained when analysing extract spiked with 0.01 mg/kg. The calibration curve is in a concentrations range from 0.001 to 0.333 µg/ml.

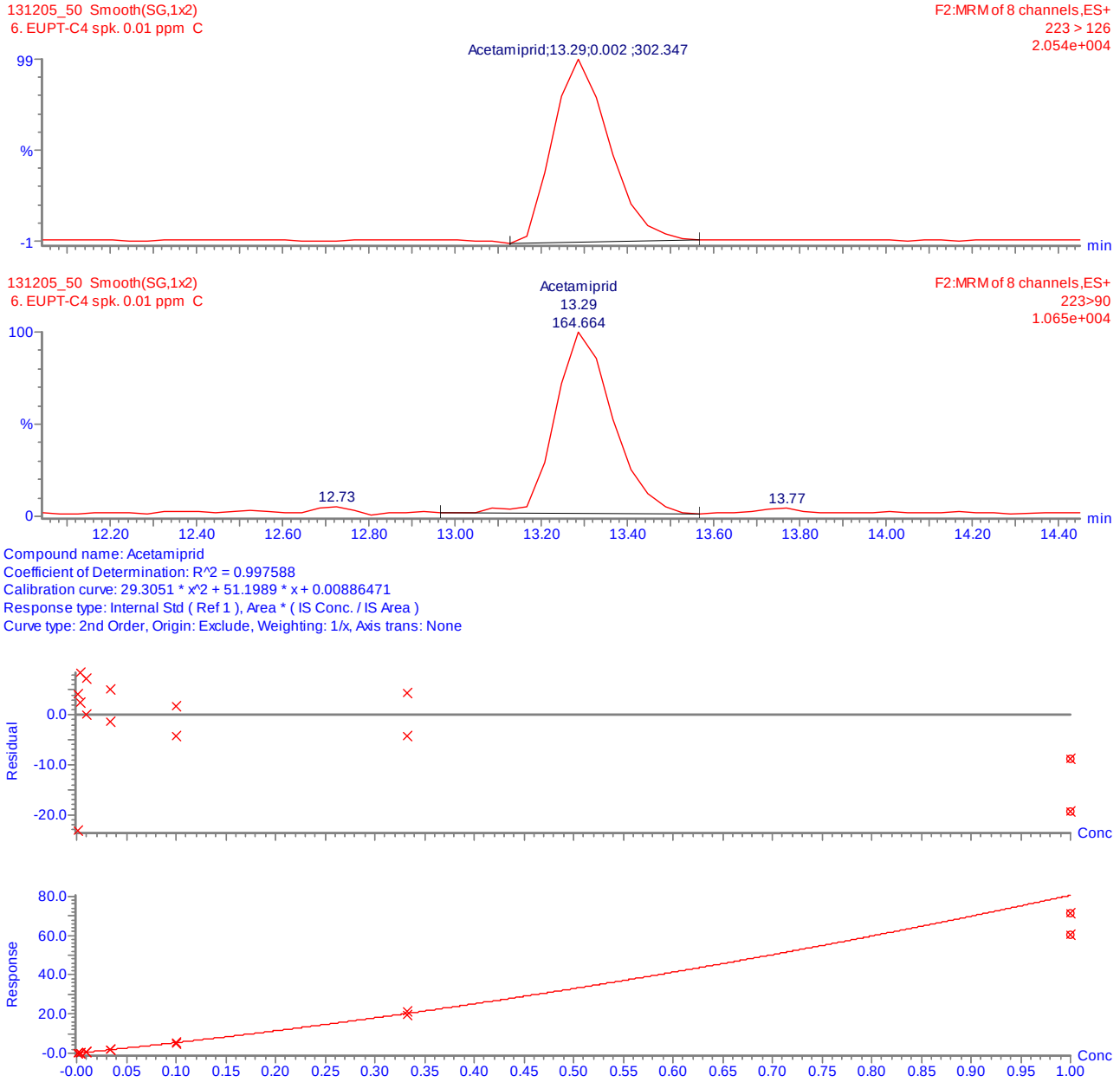

Figure 2. Examples of chromatograms, calibration curves and residual plot for acetamiprid in rye obtained when analysing extract spiked with 0.02 mg/kg. The calibration curve is in a concentrations range from 0.001 to 0.333 µg/ml.

5. Validation parameters

Precision – repeatability and internal reproducebility

Repeatability was calculated for all pesticides and degradation products on all three spiking levels. Repeatability is given as the relative standard deviation on the result from two or more analysis at the same sample, done by the same technician, on the same instrument and within a short period of time. Repeatability in this validation was calculated from the six replicate determinations. 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 recovery, repeatability and limit of quantification (LOQ) are given for the validated pesticides and degradation products for all three spiking levels (0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg). Recoveries are listed in **appendix 2-6**.

Limit of quantification, LOQ

Quantification limits (LOQ) are calculated from the results at the lowest accepted spike level, as 6 times the standard deviation (absolute recovery). The quantification limits are given in **Appendix 2**.

6. Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:

1. The relative standard deviation of the repeatability should be less than or equal to the standard deviation of 20 %, proposed for validation by Sanco[3].
2. The average relative recovery must be between 70 and 120 %[3].

If the above mentioned criteria have been meeting, the detection limits have been calculated.

7. Results and discussion

The results of the validation were intended on all four cereals; barley, rice, rye and wheat and on each individual cereal type.

For all four cereals types 51 pesticides and degradation products were validated analysed by LC-MS/MS. Nine pesticides and degradation products were not validated: Aldicarb sulfone, aldicarb sulfoxide, amitraz, DMPF, methamidophos, monocrotophos, oxamyl, thiophenat-methyl and pyridaben. However, Aldicarb sulfone were validated on barley, methamidophos on rye and thiophenat-methyl were validated on wheat. Monocrotophos and pyridaben were validated with accepteble results on two cereals types, nevertheless the validattion for all cereal type together,

the recoveries were too low and the standard deviation to high. For aldicarb sulfoxide, amitraz, DMPF and oxamyl futher investigations should be peformed.
Validations for aldicarb, diphenylamin and tolylfluanid were only accepted at spike level 0.1 mg/kg and flufenoxuron were accepted at 0.02 and 0.,1 mg/kg
The relative repeatability (RSDr) varied between 3-26 %, with an average on 9%. The internal reproducebility (RSD_R) varied between 4-28 % with an average on 12 %. Recoveries were in the range of 80-110 % at all three concentration levels with an average on 90 %. The combined LOQs were in range of 0.003-0.09 mg/kg.
For a few pesticides at some levels the standard deviations and the relative recovery were only just out of the range in proportion to criteria for acceptance. Due to the all over results at the three spiking levels for these pesticides the minor deviation were accepted.

Barley: Aldicarb sulfone, aldicarb sulfoxide, amitraz, DMPF, oxamyl, pyridaben, thiophenat-methyl, methamidophos and spinozyn D could not met the acceptance criteria and were not validated for barley. Aldicarb, diphenylamin, etofenprox, fenthion sulfon, flufenoxuron and monocrotophos were accepted at 0.1 mg/kg and fenazaquin were accepted at 0.02 and 0.1 mg/kg.
Rice: Aldicarb sulfone, aldicarb sulfoxide, amitraz, DMPF, etofenprox, fenazaquin, oxamyl pyridaben, spinozyn A, spinozyn D, spirodiclofen, thiophenat-methyl and methamidophos could not met the acceptance criteria and were not validated for rice. Diphenylamin and tolylfluanid were accepted at highest spike level and aldicarb and flufenoxuron were accepted at spike level 0.02 and 0.1 mg/kg.
Rye: Aldicarb sulfone, aldicarb sulfoxide, amitraz, DMPF, monocrotophos, oxamyl, thiophenat-methyl and tolylfluanid could not met the acceptance criteria and were not validated for rye. Aldicarb, diphenylamin, DMF, pyridaben and spirodiclofen were accepted at spike level 0.02 and 0.1 mg/kg.
Wheat: Aldicarb sulfone, aldicarb sulfoxide, amitraz, diphenylamin, DMPF, fenoxycarb, methamidophos, monocrotophos and oxamyl could not met the acceptance criteria and were not validated for wheat. Aldicarb, paraoxon-methyl and tolylfluanid were only accepted at the highest spike level.

8. Conclusions

In conclusion 51 pesticides and degradations products for levels at 0.01, 0.02 and 0.1 mg/kg were validated on barley, rice, rye and wheat using SweEt method analysed by LC-MS/MS. For rye and wheat 51 pesticides, barley 52 pesticides and rice 47 pesticides were validated.
Aldicarb sulfone, methamidophos, thiophenat-methyl, monocrotophos and pyridaben were not validated due to influence off matrix. For aldicarb sulfoxide, amitraz, DMPF and oxamyl futher investigations should be peformed.

9. References

1 Pesticide residues. Analysis in foods with ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric determination, Nordic Committee on Food Aanlysis, 13 (195);1-32

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 easurement 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.

4 EU Pesticides database available at http://ec.europa.eu/sanco_pesticides/public/index.cfm

Appendix 1: MRM transitions for the all south validated pesticides

LC-MS/MS ESI +		Retention time	Precursor 1	Product 1	CV	CE	precursor 2	Product 2	CV	CE
1	Acetamiprid	9.36	223	126	27	20		90	27	35
2	Aldicarb	11.18	213	89	29	13		116	50	12
3	Aldicarb sulfone	7.64	240.40	148	30	13		86.20	21	21
4	Aldicarb sulfoxide	7.41	224	132	10	10		89.20	21	21
5	Amitraz	24.46	294.4	163	10	16		122	10	30
6	Benfuracarb	22.16	411.60	195	30	21		252.30	30	13
7	Bitertanol	20.37	338.2	99	31	17		70	31	17
8	Bupirimat	19.35	317	166	27	23		108	25	25
9	Buprofenzin	22.33	306	201	10	11		106.2	10	23
10	Cadusafos	21.8	271.3	159	50	13		97	50	40
11	Carbendazim	10.27	192	160	55	18		132.30	30	29
12	Clofentezine	21.69	303	138	20	20		102.1	20	20
13	Dimethomorph	16.73/17.48	388	301	45	20		165	23	30
14	Diphenylamine	19.35	170	93.1	25	20		151.9	25	20
15	DMF	12.5	150.25	132.2	34	35		106.8	34	20
16	DMPF	8.73	163.715	122.955	10	20		132.936	10	20
17	DMST	13.8	232	106	30	20		150.9	30	20
18	Ethoprophos	19.28	243	97	50	23		131	50	31
19	Etofenprox	26.83	394	177.3	25	20		107.1	25	20
20	Fenamiphos	18.93	304	217.1	31	23		202	31	33
21	Fenamiphos sulfoxide	12.88	320	171	50	13		292	50	40
22	Fenamiphos sulfone	13.37	336	188	50	31		266	50	23
23	Fenazaquin	25.07	307	161.2	55	17		131.1	52	14
24	Fenoxycarb	20.29	302.3	88.2	42	31		116.2	42	31

	LC-MS/MS ESI +	Retention time	Precursor 1	Product 1	CV	CE	precursor 2	Product 2	CV	CE
25	Fenthion	20.96	279	169.1	25	20		247.1	25	20
26	Fenthion oxon	17.03	263	231	20	30		216	20	15
27	Fenthion oxon-sulfone	9.8	295	217	33	20		78	33	20
28	Fenthion oxon-sulfoxide	9.41	279	264	20	15		104	20	20
29	Fenthion sulfoxide	13.61	295	279.7	25	15		108.9	25	20
30	Fenthion sulfone	14.24	311	125	25	20		108.9	25	20
31	Flufenoxuron	24.06	489.6	158.1	22	13		141	22	40
32	Hexythiazox	22.78	353	228	45	11		168	45	27
33	Iprovalicarb	17.76	321	119.1	45	17		91.2	45	48
34	Lufenuron	22.43	511	158.1	33	21		141	33	41
35	Mepanipyrim	18.39	224	106.1	17	23		77.27	17	38
36	Metalaxyl	14.42	280	220.1	52	11		160.1	52	22
37	Methamidophos	6.45	142	94	40	12		124.90	40	12
38	Methiocarb	17.36	243.40	169.3	30	13		121.20	30	21
39	Methiocarb sulfone	9.59	275	122	33	25		107.20	33	40
40	Methiocarb sulfoxide	8.73	242	185	33	10		122	33	30
41	Monocrotophos	8.13	241	127	21	10		193	10	20
42	Oxadixyl	10.72	279	219.1	17	17		132.1	17	30
43	Oxamyl	7.64	237.40	72.4	21	13		90.30	21	8
44	Paraoxon-methyl	11.23	265	202	51	35		127	40	19
45	Phosmet	15.89	335	160	17	17		133	17	45
46	Phosmet oxon	11.1	302	160.2	29	21		133.2	29	35
47	Phoxim	21.27	299	77.1	10	23		115	17	17
48	Propargite	23.18	368	175.1	24	15		231	24	11
49	Pyridaben	24.36	365	147.2	38	23		309.1		
50	Pyriproxyfen	22.89	322	96	55	20		185	27	23

LC-MS/MS ESI +		Retention time	Precursor 1	Product 1	CV	CE	precursor 2	Product 2	CV	CE
51	Spinosyn A	21.72	733	142	30	20		98	30	20
52	Spinosyn D	22.59	746.5	142	30	40		98.2	30	45
53	Spirodiclofen	24.39	411	313	20	20		71	20	20
54	Tebufenpyrad	22.08	334.5	147	55	23		117	55	30
55	Thiabendazole	11.24	201.80	175	45	24		131.20	45	29
56	Thiofenat methyl	12.59	343	151	50	20		93	50	40
57	Tolylfluanid	19.91	347	137	40	25	364	238	40	25
58	Trichlorfon	9.88	256.9	109	34	15		221	34	15
59	Triflumuron	20.57	359	155.9	21	25		139	20	30
60	Zoxamide	21.21	336.4	187	36	40		159	36	20

Appendix 2: Recoveries, repeatability (RSDr), reproduceability (RSD_R) and Limit of Quantification (LOQ) for pesticides validated on barley, rice, rye and wheat.

Numbers in italic is outside 70-120% recovery

SweEt – barley, rice, rye and wheat	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, %	**RSD_r, %**	**RSD_R, %**		**Recovery, %**	**RSD_r, %**	**RSD_R, %**		**Recovery, %**	**RSD_r, %**	**RSD_R, %**		**LOQ**
Acetamiprid	91	5	6		92	8	10		85	3	9		0.003
Aldicarb									91	13	13		0.07
Benfuracarb	96	10	15		87	12	17		93	7	17		0.008
Bitertanol	86	7	11		88	11	12		90	4	8		0.005
Bupirimate	95	4	15		92	6	14		87	4	6		0.008
Buprofezin	96	14	14		86	8	17		83	4	18		0.008
Cadusafos	89	6	11		89	15	17		91	12	13		0.005
Carbendazim	88	12	11		87	12	18		81	6	19		0.006
Clofentezine	86	13	13		81	10	12		81	7	11		0.007
Dimethomorph	91	4	7		96	8	8		93	3	4		0.004
Diphenylamin									93	9	10		0.08
DMF	82	14	23		94	10	16		93	5	15		0.02
DMST	89	13	26		90	12	20		87	7	8		0.01
Ethoprophos	92	6	10		97	9	12		91	4	9		0.005
Etofenprox	93	11	10		90	10	13		84	5	23		0.02
Fenamiphos	86	6	9		86	19	20		83	6	10		0.004
Fenamiphos sulfoxide	89	13	16		95	14	16		90	5	5		0.008
Fenamiphos sulfone	92	8	8		102	9	11		100	4	5		0.005
Fenazaquin	88	6	6		83	10	15		84	4	17		0.01
Fenoxycarb	84	11	17		86	14	14		87	6	9		0.007
Fenthion	81	15	18		85	15	23		92	7	10		0.008
Fenthion oxon	87	5	10		94	9	14		91	4	7		0.005

SweEt – barley, rice, rye and wheat	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, %	**RSD_r, %**	**RSD_R, %**		**Recovery, %**	**RSD_r, %**	**RSD_R, %**		**Recovery, %**	**RSD_r, %**	**RSD_R, %**		**LOQ**
Fenthion oxon-sulfone	91	16	*23*		104	13	18		101	7	9		0.01
Fenthion oxon-sulfoxide	84	5	8		92	9	10		89	4	4		0.003
Fenthion sulfone	96	12	*27*		89	14	18		88	8	11		0.02
Fenthion sulfoxide	100	8	12		100	10	13		97	5	6		0.007
Flufenoxuron					97	*26*	*28*		83	18	18		0.04
Hexythiazox	93	15	19		86	11	13		82	10	18		0.01
Iprovalicarb	87	10	11		91	11	12		85	8	9		0.006
Lufenuron	87	13	*21*		86	11	15		88	8	10		0.01
Mepanipyrim	96	9	16		94	14	18		91	6	12		0.009
Metalaxyl	98	7	12		97	10	14		93	3	7		0.006
Methiocarb	83	11	11		92	11	14		93	4	4		0.006
Methiocarb sulfone	94	6	7		93	9	9		92	3	5		0.004
Methiocarb sulfoxide	91	7	9		85	9	9		79	4	7		0.005
Oxadixyl	88	19	*21*		98	11	14		97	6	8		0.01
Paraoxon-methyl	81	20	19		95	16	19		91	4	17		0.01
Phosmet	89	9	11		90	11	14		92	3	10		0.006
Phosmet oxon	95	4	5		98	7	9		93	3	5		0.003
Phoxim	89	8	10		88	7	9		85	3	5		0.005
Propargite	94	6	8		88	10	14		89	7	14		0.004
Pyriproxyfen	83	4	8		78	8	8		79	3	7		0.004
Spinozyn A	83	12	13		81	11	15		78	5	14		0.006
Spinozyn D	86	18	*26*		88	16	17		81	7	11		0.01
Spirodiclofen	103	18	*28*		90	9	17		89	5	14		0.01
Tebufenpyrad	92	5	9		88	8	13		85	3	9		0.004
Thiabendazole	87	5	6		88	9	10		89	11	12		0.003
Tolylfluanid									108	5	11		0.09

SweEt – barley, rice, rye and wheat	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, %	**RSD_r, %**	**RSD_R, %**		**Recovery, %**	**RSD_r, %**	**RSD_R, %**		**Recovery, %**	**RSD_r, %**	**RSD_R, %**		**LOQ**
Trichlorfon	89	8	10		95	11	17		93	5	13		0.005
Triflumuron	87	6	7		92	9	10		87	4	5		0.004
Zoxamide	85	18	*23*		89	17	16		90	5	5		0.01

Appendix 3: Recoveries, repeatability (RSDr) and Limit of Quantification (LOQ) for pesticides validated on barley.

Barley - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Acetamiprid	91	5		86	4		79	4		0.002
Aldicarb							91	9		0.05
Aldicarb sulfone	77	12		77	8		75	4		0.005
Benfuracarb	102	4		95	15		96	4		0.003
Bitertanol	80	10		84	9		93	3		0.005
Bupirimate	83	6		86	1		90	4		0.003
Buprofezin	95	2		75	9		77	4		0.001
Cadusafos	89	2		95	4		98	6		0.001
Carbendazim	89	5		80	2		70	3		0.003
Clofentezine	84	9		84	2		81	6		0.004
Dimethomorph	86	4		92	3		92	3		0.002
Diphenylamin					15		91	9		0.05
DMF	75	16		91	9		93	7		0.007
DMST	80	18		82	9		83	9		0.009
Ethoprophos	83	9		86	7		80	4		0.005
Etofenprox							85	8		0.04
Fenamiphos	81	4		90	15		88	5		0.002
Fenamiphos sulfoxide	82	19		84	14		89	7		0.009
Fenamiphos sulfone	92	10		98	5		97	4		0.006
Fenazaquin				74	8		81	4		0.007
Fenoxycarb	72	8		80	7		86	4		0.003
Fenthion	84	10		90	5		97	5		0.005
Fenthion oxon	83	5		91	3		89	2		0.002
Fenthion oxon-sulfone	103	19		107	9		106	8		0.01
Fenthion oxon-sulfoxide	78	5		89	4		88	3		0.003

Barley - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Fenthion sulfone							91	7		0.04
Fenthion sulfoxide	105	3		99	4		97	4		0.002
Flufenoxuron							72	11		0.05
Hexythiazox	79	16		77	7		78	5		0.008
Iprovalicarb	81	14		94	6		88	11		0.007
Lufenuron	78	14		83	9		84	11		0.007
Mepanipyrim	90	6		94	6		97	5		0.003
Metalaxyl	102	3		97	3		93	4		0.002
Methiocarb	79	6		79	6		93	2		0.003
Methiocarb sulfone	95	6		95	4		95	2		0.003
Methiocarb sulfoxide	92	5		82	3		73	3		0.003
Monocrotophos							74	7		0.01
Oxadixyl	97	6		91	8		92	6		0.004
Paraoxon-methyl	83	8		84	12		*93*	4		0.004
Phosmet	88	6		90	4		97	2		0.003
Phosmet oxon	95	6		97	3		95	3		0.003
Phoxim	87	7		87	5		83	1		0.004
Propargite	87	6		83	3		89	4		0.003
Pyriproxyfen	76	5		78	3		81	3		0.002
Spinozyn A	75	9		70	10		68	5		0.004
Spirodiclofen	83	12		76	11		76	4		0.006
Tebufenpyrad	92	4		87	7		87	3		0.002
Thiabendazole	84	9		82	4		84	4		0.004
Tolylfluanid				90	*22*		104	6		0.02
Trichlorfon	89	5		96	6		88	4		0.002
Triflumuron	85	7		89	2		88	3		0.004
Zoxamide	79	18		88	6		89	2		0.008

Appendix 4: Recoveries, repeatability (RSDr) and Limit of Quantification (LOQ) for pesticides validated on rice.

Rice - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Acetamiprid	93	5		92	3		79	5		0.003
Aldicarb				98	*22*		85	14		0.02
Benfuracarb	91	3		73	4		74	10		0.002
Bitertanol	96	6		91	8		81	6		0.004
Bupirimate	106	2		107	4		91	4		0.001
Buprofezin	95	2		74	3		*66*	5		0.001
Cadusafos	87	3		79	6		86	13		0.002
Carbendazim	86	5		76	4		*66*	3		0.003
Clofentezine	82	5		75	7		72	11		0.003
Dimethomorph	92	4		100	5		91	3		0.002
Diphenylamin							89	14		0.03
DMF	73	20		77	4		82	5		0.009
DMST	78	16		79	6		92	7		0.007
Ethoprophos	95	2		101	6		94	4		0.001
Fenamiphos	81	4		77	8		76	8		0.002
Fenamiphos sulfoxide	92	9		106	13		90	4		0.005
Fenamiphos sulfone	89	9		112	6		104	5		0.005
Fenoxycarb	87	5		88	6		94	9		0.002
Fenthion	77	11		79	8		85	9		0.005
Fenthion oxon	93	5		105	6		96	5		0.003
Fenthion oxon-sulfone	77	17		116	17		101	5		0.008
Fenthion oxon-sulfoxide	87	5		101	5		91	6		0.002
Fenthion sulfone	80	12		75	11		78	11		0.006

Rice - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Fenthion sulfoxide	88	4		87	6		91	4		0.002
Flufenoxuron				81	*29*		89	19		0.03
Hexythiazox	100	17		86	12		*67*	17		0.01
Iprovalicarb	93	4		93	2		80	5		0.002
Lufenuron	*69*	*21*		82	7		81	7		0.009
Mepanipyrim	86	6		75	5		76	9		0.003
Metalaxyl	84	4		82	2		84	3		0.002
Methiocarb	89	9		99	6		92	6		0.005
Methiocarb sulfone	89	4		88	4		87	4		0.002
Methiocarb sulfoxide	84	6		83	4		82	2		0.003
Monocrotophos	92	11		89	*22*		75	9		0.006
Oxadixyl	75	*32*		112	6		104	8		0.01
Paraoxon-methyl	76	*27*		109	11		107	6		0.01
Phosmet	98	4		91	7		93	2		0.003
Phosmet oxon	98	3		100	5		90	4		0.002
Phoxim	89	5		84	5		81	4		0.003
Propargite	97	8		77	3		74	7		0.005
Pyriproxyfen	79	4		73	5		72	4		0.002
Tebufenpyrad	95	6		91	3		77	4		0.004
Thiabendazole	89	2		90	3		83	5		0.001
Tolylfluanid							120	3		0.04
Trichlorfon	91	6		113	4		108	6		0.007
Triflumuron	91	4		96	3		84	4		0.002
Zoxamide	73	13		84	12		91	7		0.006

Appendix 5: Recoveries, repeatability (RSDr) and Limit of Quantification (LOQ) for pesticides validated on rye.

Rye - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Acetamiprid	93	6		100	3		94	1		0.004
Aldicarb				93	13		92	12		0.02
Benfuracarb	82	10		85	2		96	4		0.005
Bitertanol	89	4		94	7		94	2		0.002
Bupirimate	107	4		97	4		84	2		0.003
Buprofezin	103	*23*		94	4		94	3		0.01
Cadusafos	100	8		98	5		96	4		0.005
Carbendazim	91	*21*		103	3		96	4		0.01
Clofentezine	92	18		88	3		89	5		0.01
Dimethomorph	98	5		99	3		93	1		0.003
Diphenylamin				104	19		98	3		0.04
DMF				101	8		87	5		0.02
DMST	79	10		88	11		83	4		0.005
Ethoprophos	102	6		106	3		95	1		0.004
Etofenprox	90	12		84	14		70	5		0.006
Fenamiphos	93	9		97	2		89	2		0.005
Fenamiphos sulfoxide	101	11		99	11		91	3		0.007
Fenamiphos sulfone	97	7		104	6		100	1		0.004
Fenazaquin	87	8		82	5		72	5		0.004
Fenoxycarb	93	16		90	11		83	4		0.009
Fenthion	93	*21*		105	6		98	5		0.01
Fenthion oxon	93	4		100	4		94	3		0.002
Fenthion oxon-sulfone	107	13		110	6		104	8		0.009
Fenthion oxon-sulfoxide	83	6		89	2		88	1		0.003
Fenthion sulfone	*124*	10		98	12		91	7		0.008

Rye - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Fenthion sulfoxide	99	8		102	4		100	2		0.005
Flufenoxuron	119	*34*		109	7		84	20		0.02
Hexythiazox	105	15		88	4		88	5		0.01
Iprovalicarb	92	11		96	10		88	9		0.006
Lufenuron	102	9		97	10		91	5		0.005
Mepanipyrim	115	11		107	3		97	3		0.008
Metalaxyl	107	11		105	4		97	2		0.007
Methamidophos	100	18		93	10		77	3		0.01
Methiocarb	81	17		95	4		91	2		0.008
Methiocarb sulfone	97	7		97	3		94	2		0.004
Methiocarb sulfoxide	99	9		90	5		81	1		0.005
Oxadixyl	93	15		97	4		96	4		0.009
Paraoxon-methyl	83	*21*		93	10		94	3		0.01
Phosmet	89	15		100	4		98	2		0.008
Phosmet oxon	96	4		104	4		98	1		0.002
Phoxim	97	11		95	7		86	3		0.006
Propargite	95	4		98	3		92	11		0.002
Pyridaben				106	6		83	8		0.02
Pyriproxyfen	87	5		81	5		77	1		0.002
Spinozyn A	87	13		87	4		77	5		0.007
Spinozyn D	98	*21*		92	7		76	7		0.01
Spirodiclofen				100	4		98	4		0.01
Tebufenpyrad	99	4		98	7		94	3		0.002
Thiabendazole	89	6		93	4		97	19		0.003
Trichlorfon	95	11		96	8		95	3		0.006
Triflumuron	90	7		97	10		92	4		0.004
Zoxamide	82	*25*		91	14		87	4		0.01

Appendix 6: Recoveries, repeatability (RSDr) and Limit of Quantification (LOQ) for pesticides validated on wheat.

Wheat - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Acetamiprid	85	2		89	4		88	2		0.001
Aldicarb							94	16		0.09
Benfuracarb	109	15		96	9		108	10		0.01
Bitertanol	79	6		85	8		81	6		0.003
Bupirimate	83	4		79	4		83	5		0.002
Buprofezin	89	15		101	11		96	3		0.008
Cadusafos	80	7		86	12		86	20		0.003
Carbendazim	86	9		90	4		91	8		0.004
Clofentezine	81	7		77	14		84	7		0.004
Dimethomorph	87	5		93	5		97	4		0.002
Etofenprox	95	9		*96*	6		*97*	5		0.005
Fenamiphos sulfoxide	79	12		89	10		91	5		0.006
Fenamiphos sulfone	88	4		96	4		101	4		0.002
Fenazaquin	90	2		93	3		99	4		0.001
Fenthion	71	9		*68*	7		87	8		0.004
Fenthion oxon	79	7		80	6		85	5		0.003
Fenthion oxon-sulfoxide	*88*	4		90	4		89	3		0.002
Fenthion sulfone	85	14		91	8		92	8		0.007
Fenthion sulfoxid	109	12		110	5		99	8		0.008
Flufenoxuron	108	10		100	9		86	19		0.006
Hexythiazox	88	9		94	9		96	12		0.005
Iprovalicarb	82	7		82	6		82	5		0.003
Lufenuron	97	8		87	2		81	7		0.005
Mepanipyrim	93	9		94	7		95	5		0.005
Metalaxyl	99	3		102	6		97	4		0.002

Wheat - SweEt	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 %**		**Recovery %**	**RSDr %**		**Recovery %**	**RSDr %**		**LOQ**
Methiocarb	85	7		94	12		96	5		0.004
Methiocarb sulfone	97	5		93	4		94	5		0.003
Methiocarb sulfoxide	89	5		87	8		81	8		0.002
Oxadixyl	90	7		92	14		97	6		0.004
Paraoxon-methyl							72	4		0.02
Phosmet	81	7		79	5		79	5		0.003
Phosmet oxon	91	3		89	3		91	4		0.002
Phoxim	84	5		87	3		90	3		0.003
Propargite	95	6		93	6		102	5		0.004
Pyridaben	89	5		94	6		98	5		0.003
Pyriproxyfen	89	4		80	1		85	4		0.002
Spinozyn A	85	13		85	7		89	4		0.007
Spinozyn D	74	10		83	6		85	8		0.004
Spirodiclofen	98	10		95	9		94	6		0.006
Tebufenpyrad	83	6		76	5		81	4		0.003
Thiabendazole	84	3		87	4		92	3		0.002
Thiophenat-Methyl	83	7		71	15		76	19		0.004
Tolylfluanid							100	5		0.03
Trichlorfon	79	5		77	5		81	3		0.002
Triflumuron	83	5		85	6		85	4		0.002
Zoxamide	106	13		90	14		93	3		0.008

Appendix 7: Flow diagram for SweEt method - cereals

In a 50 ml centrifuge tube with screw cap: Weigh 5 g grinded sample
Add 10 ml water, 10 ml ethyl acetate with 1 % acetic acid and 10 g sodium sulphate
Shake vigorously (Vortex) approximately 30 seconds and extract the sample for 20 minutes in an ultrasonic bath, max 35ºC
Centrifuge in 3 minutes at 3200 g
Filter the organic face true 0.2 µm syringe filters
Analysis by GC-MS/MS and LC-MS/MS