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Appendix 3

Validation Report 11

Determination of Pesticide Residues in wheat by GC-MS/MS

SweEt method

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CONTENT

1 Introduction

This report describes the validation of the SweEt method combined with GC-MS/MS. The method is a Swedish developed method based on simple and efficient process with ethyl acetate as extraction solvent. This validation does not include testing of the methods robustness.
The method was south validated for 67 pesticides and degradation products in wheat.

2 Principle of analysis

2.1 Sample preparation

The sample was grinded and sifted with an 1.0 mm sieve

2.2 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.

2.3 Clean-up

The method does not include any clean-up step.

2.4 Quantification and qualification

The final extract was analysed by GC-MS/MS (electron energy 70eV, source temp. 180°C, transfer line GC interface 250°C) with an injection volume of 5 µl.

2.5 Selectivity and specificity

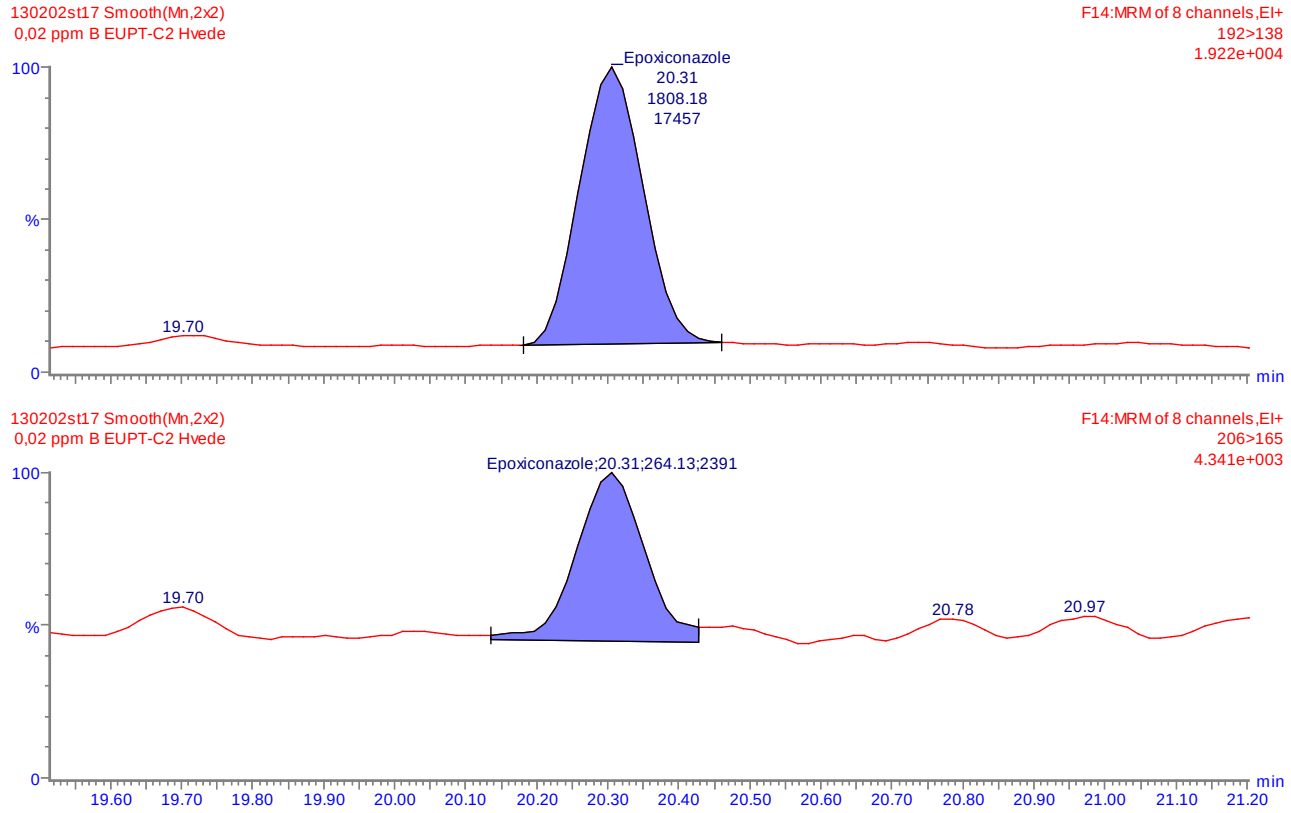
GC-MS/MS is a highly selective method, and thereby highly specific. All pesticides were detected in the Multi Reaction Monitoring mode (MRM). For each pesticide two precursor ion and two product ions (where possible) were determined - one product ion for quantification and one for qualification. The MRM transitions for the pesticides and degradation products indented validated are given in appendix 1.

3 Validation design

The method was south validated for 67 pesticides or degradation products in 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. A blank sample of wheat was included. The tests were done on same day with six replicates by the same person.

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 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 and calibration curves are presented in figure 1 and 2, respectively.



Compound name: Epoxiconazole
Correlation coefficient: r = 0.999193, r^2 = 0.998387
Calibration curve: 400948 * x + 220.276
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

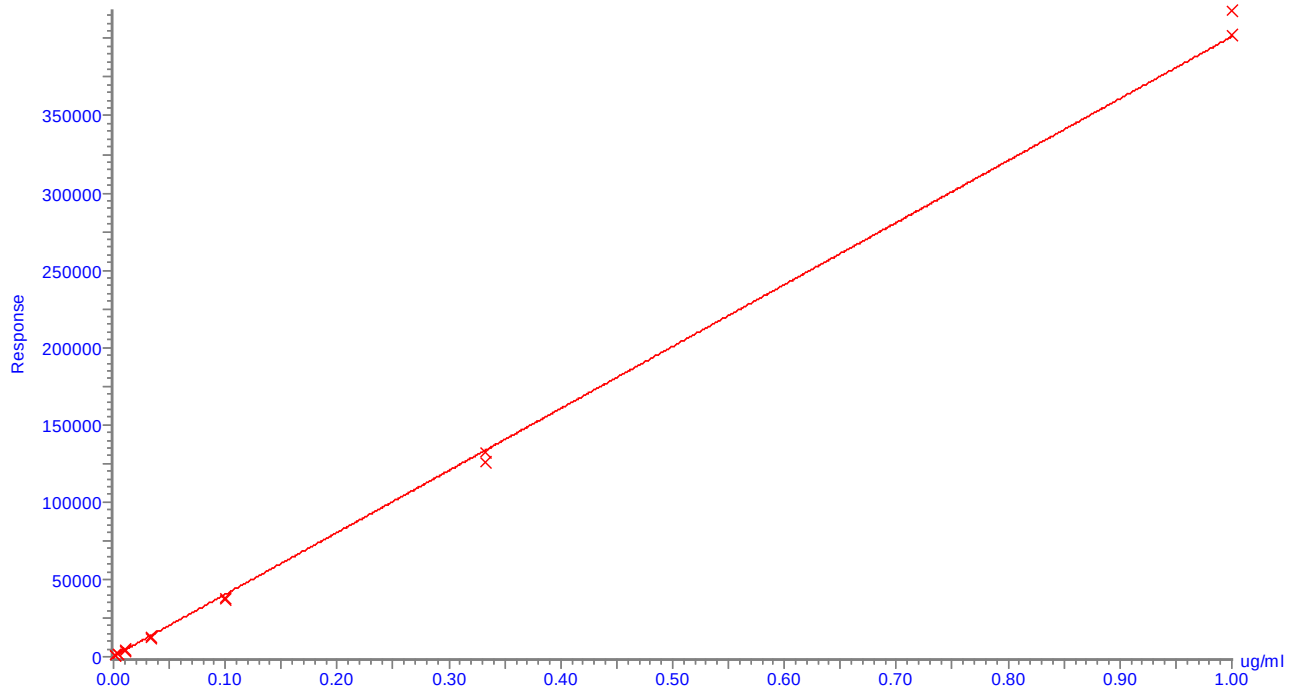


Figure 1 Examples of chromatograms and calibration curves for epoxiconazole in wheat obtained when analysing extract spiked with 0.10 mg/kg. (Two MRM transitions are shown for epoxiconazole) The calibration curve is in a concentrations range from 0.003 to 0.333 µg/ml.

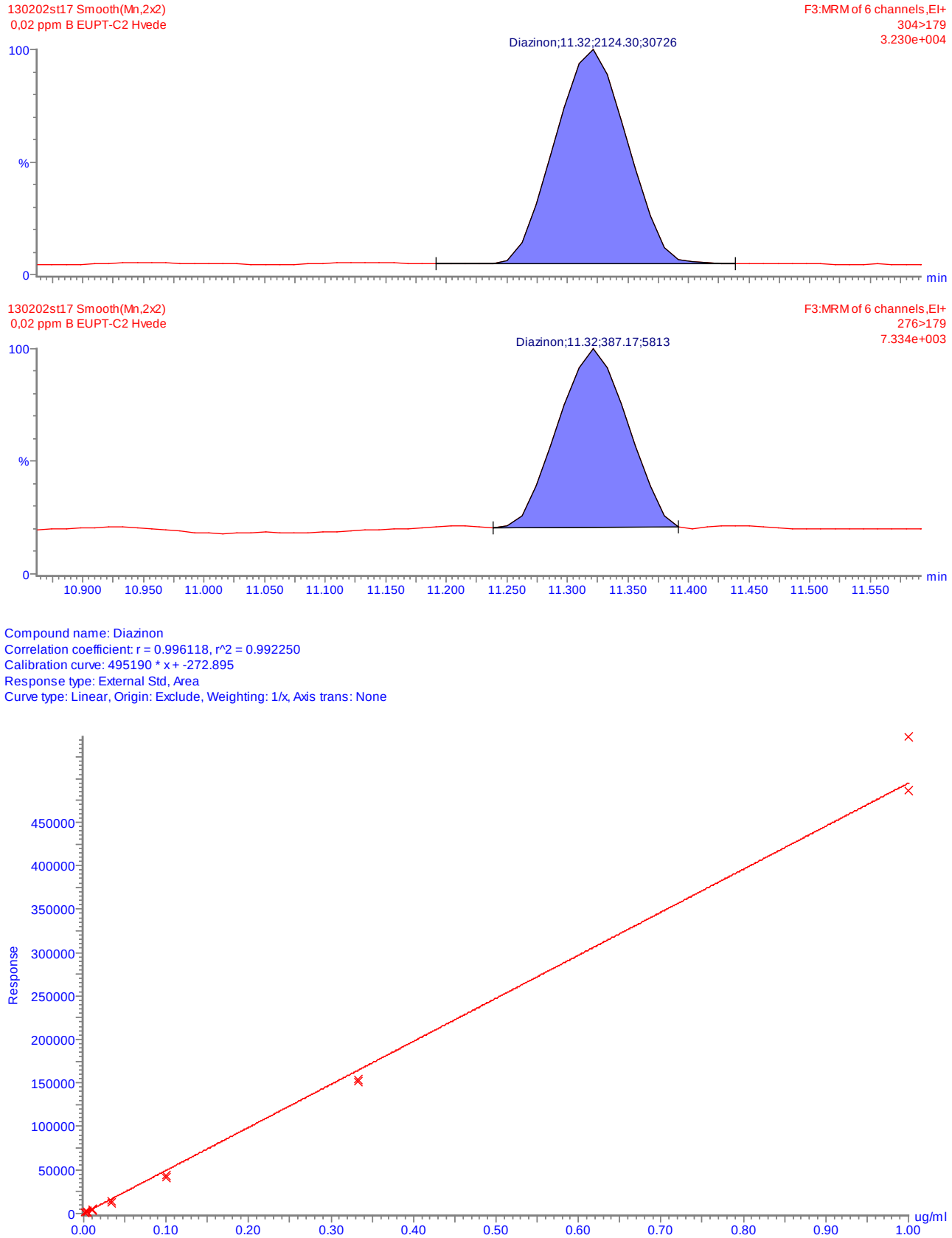


Figure 2 Examples of chromatograms and calibration curves for diazinon in wheat obtained when analysing extract spiked with 0.02 mg/kg. (Two MRM transitions are shown for diazinon) The calibration curve is in a concentrations range from 0.003 to 0.333 µg/ml.

5 Validation parameters

5.1 Precision – repeatability

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. Repeatability was calculated as given in ISO 5725-2[2].

Appendix 2 shows the relative repeatability for the validated pesticides and degradation products.

5.2 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.

5.3 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 must be less than or equal to the standard deviation proposed by Horwitz[3].
2. The average relative recovery must be between 70 and 120 %[4].

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

7 Results and discussion

The SweEt method has been tested for 67 pesticides and degradations products in wheat using GC-MS/MS. For spikes levels at 0.01, 0.02 and 0.1 mg/kg 52 pesticides were validated. Validations for Dimethoate, Endosulfan sulphate, Endosulfan-alpha, Endosulfan-beta, Deltamethrin (cis) and Chlorpropham were only accepted at spike level 0.1 mg/kg. Endosulfan-alpha was not approved at

the low levels probably due to matrix effect. This could maybe be eliminated by choosing other transitions.

The relative repeatability (RSDr) varied between 2-30 %, however for most the values were below 10 %. For the majority of the pesticides the recovery was in range of 80-110 % at all three concentrations levels.

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.

The combined LOQs were in range of 0.01-0.02 mg/kg, although for Dimethoate, Endosulfan sulphate, Endosulfan-alpha, Endosulfan-beta, Deltamethrin (cis) and Chlorpropham the LOQs were calculated higher (0.12-0.19 mg/kg).

8 Conclusions

In conclusion 58 pesticides and degradations products for levels at 0.01, 0.02 and 0.1 mg/kg were validated on wheat SweEt method using GC-MS/MS for the analysis. Six pesticides, Dimethoate, Endosulfan sulphate, Endosulfan-alpha, Endosulfan-beta, Deltamethrin (cis) and Chlorpropham, were only accepted at spike level 0.1 mg/kg.

9 References

1 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.

2 W. Horwitz, Anal. Chem., 1982; 54, 67A.

3 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/10684/2010, 01/01/2010, European Commission, Brussels, 2010.

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 (Samstik A)

GC-MS/MS		Precursor 1	Fragment ion 1	Col. energy 1	Precursor 2	Fragment ion 2	Col. energy 2
1	Azinphos-methyl	160	77	15	132	77	10
2	Azoxystrobin	344	329	15	388	345	15
3	Bifenthrin	181	166	10	165	115	20
4	Boscalid	342	140	15	167	139	20
5	Captan	149	70	12	149	105	2
6	Carbofuran	149	121	5	164	149	10
7	Carboxin	235	143	5	143	87	5
8	Chlorfenvinphos	323	267	15	295	267	5
9	Chlorothanlonil	266	133	18	266	231	10
10	Chlorpropham	213	127	15	213	171	5
11	Chlorpyrifos	197	169	10	314	258	12
12	Chlorpyrifos-methyl	286	93	20	125	79	5
13	Cyfluthrin-total	226	206	10	163	91	10
14	Cypermethrin-total	163	127	10	181	152	20
15	Cyproconazole	222	125	15	139	111	15
16	Cyprodinil	226	225	15	223	208	15
17	Deltamethrin-cis	181	152	10	253	174	10
18	Diazinon	304	179	10	276	179	10
19	Dichlorvos	109	79	5	187	93	10
20	Difenoconazole	323	265	15	325	267	15
21	Dimethoate	229	87	7	125	79	6
22	Endosulfan sulfate	272	236	20	387	252	10
23	Endosulfan α	195	159	5	339	159	20
24	Endosulfan β	195	159	5	339	159	20
25	Epoxiconazole	192	138	10	206	165	5
26	Ethion	384	231	5	231	203	15
27	Fenbuconazole	198	129	10	129	102	15
28	Fenitrothion	277	260	5	277	109	15
29	Fenpropidin	98	70	10	99	71	10
30	Fenpropimorph	303	128	5	117	115	10
31	Fenvalerate	167	125	10	125	99	10
32	Fipronil	367	213	20	367	255	15

GC-MS/MS		Precursor 1	Fragment ion 1	Col. energy 1	Precursor 2	Fragment ion 2	Col. energy 2
33	Fluquinconazole	340	298	15	339	298	15
34	Flutriafol	219	123	15	123	95	10
35	HCH alpha	217	181	10	219	183	10
36	HCH beta	217	181	10	219	183	10
37	Hexaconazole	231	175	10	214	172	15
38	Iprodione	314	245	10	216	187	5
39	Isoprothiolane	290	118	10	290	204	2
40	Kresoxim-methyl	206	116	4	206	131	10
41	Lambda-cyhalothrin	197	141	10	208	181	10
42	Lindane	217	181	10	219	183	10
43	Malathion	173	99	10	173	127	5
44	Metconazole	125	89	10	127	89	10
45	Metribuzin	198	82	15	214	198	5
46	2-phenylphenol	141	115	15	170	169	10
47	Parathion	291	109	10	291	81	20
48	Penconazole	248	157	20	159	123	15
49	Pendimethalin	281	252	5	252	162	5
50	Permethrin	183	168	15	183	153	10
51	Phosphamidone	264	127	10	127	109	10
52	Pirimicarb	238	166	10	166	96	10
53	Pirimiphos-methyl	305	290	10	290	233	10
54	Prochloraz	180	138	10	310	268	5
55	Procymidone	283	96	6	283	254	10
56	Propiconazole	173	145	15	259	173	15
57	Pyrimethanil	199	198	5	198	183	10
58	Quinoxyfen	237	208	20	272	237	15
59	Tebuconazole	250	125	15	125	89	10
60	Thiamethoxam	212	139	10	247	182	10
61	Triadimefon	208	181	5	181	111	10
62	Triadimenol	168	70	5	128	100	10
63	Triazophos	257	162	5	285	162	10
64	Trifloxystrobin	222	190	5	186	145	10
65	Trifluralin	264	206	5	290	248	10
66	Triticonazole	235	182	15	217	167	15
67	Vinclozolin	285	212	5	198	145	15

Appendix 2: Recoveries, repeatability (RSDr) and Limit of Quantification (LOQ) for pesticides validated on wheat

SweEt - Wheat	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, %	**RSD_r %**		**Recovery, %**	**RSD_r %**		**Recovery, %**	**RSD_r %**	**LOQ**
Azoxystrobin	79	21		82	9		71	5	0.010
Bifenthrin	89	6		82	2		74	5	0.003
Boscalid	81	11		73	6		67	5	0.005
Chlorfenvinphos	101	6		98	2		86	4	0.004
Chlorpropham							93	11	0.012
Chlorpyrifos	105	4		109	6		88	4	0.002
Chlorpyrifos-methyl	113	5		105	4		79	5	0.003
Cyfluthrin-total	65	20		76	9		77	7	0.008
Cypermethrin-total	97	8		80	10		72	4	0.004
Cyproconazole	89	3		95	5		87	5	0.002
Cyprodinil	120	17		119	10		85	7	0.012
Deltamethrin (cis)							86	8	0.018
Diazinon	115	5		95	3		68	4	0.004
Difenoconazole	78	13		77	7		67	5	0.006
Dimethoate							75	8	0.195
Endosulfan sulfate							73	20	0.027
Endosulfan-alpha							92	9	0.039
Endosulfan-beta							79	12	0.076
Epoxiconazole	82	6		80	2		77	5	0.003
Ethion	112	5		94	2		70	2	0.003
Fenbuconazole	92	14		83	7		72	4	0.008
Fenitrothion	110	5		100	2		77	3	0.003
Fenpropidin	104	11		103	8		91	3	0.007
Fenpropimorph	111	5		88	8		76	3	0.004

SweEt - Wheat	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, %	**RSD_r %**		**Recovery, %**	**RSD_r %**		**Recovery, %**	**RSD_r %**	**LOQ**
Fenvalerate RS-SR							84	8	0.004
Fipronil	104	6		95	5		74	2	0.004
Fluquinconazole	86	14		82	4		71	6	0.007
Flutriafol	130	9		109	6		94	5	0.007
HCH - alpha	105	11		112	8		99	4	0.007
HCH - beta	111	2		108	5		85	2	0.001
Hexaconazole	94	15		101	12		94	6	0.008
Iprodione	79	13		110	4		81	6	0.006
Isoprothiolane	98	6		97	3		79	6	0.004
Kresoxim-methyl	83	7		92	3		78	7	0.004
Lambda-cyhalothrin	102	19		99	5		90	4	0.012
Lindane	112	3		111	7		85	2	0.002
Malathion	71	12		71	3		77	6	0.005
Metconazole	75	19		73	15		81	7	0.009
Metribuzin	77	18		89	8		74	5	0.025
Parathion	108	6		102	4		80	4	0.004
Pendimethalin	109	8		104	10		77	6	0.005
Permethrin	107	25		90	18		90	5	0.016
Phosphamidone	106	12		95	7		76	5	0.008
Pirimicarb	120	3		117	2		81	6	0.002
Pirimiphos-methyl	117	4		100	7		71	5	0.003
Procymidone	91	10		99	4		83	3	0.005
Propiconazole	80	30		82	13		73	5	0.015
Pyrimethanil	109	6		88	2		72	4	0.004
Quinoxyfen	103	2		101	3		83	4	0.001
Tebuconazole	80	10		80	7		79	6	0.005
Thiamethoxam	73	30		75	10		69	5	0.013

SweEt - Wheat	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, %	**RSD_r %**		**Recovery, %**	**RSD_r %**		**Recovery, %**	**RSD_r %**	**LOQ**
Triadimefon	89	12		74	4		80	5	0.006
Triadimenol	85	21		70	11		84	4	0.011
Triazophos	98	11		87	5		70	2	0.007
Trifloxystrobin	113	24		109	22		79	4	0.019
Trifluralin	119	6		109	7		80	4	0.004
Triticonazole	108	18		109	8		88	7	0.012
Vinclozolin	113	19		105	7		74	4	0.013

Appendix 3: Flow diagram for SweEt method