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National Food Institute
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Validation Report 30

Validation of quantification method for the analysis of pesticide residues in cereals by using Exactive™ GC Orbitrap™ GC-MS system from Thermo Fisher Scientific

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1. Introduction

This report describes the validation of the QuEChERS method combined with Exactive™ GC Orbitrap™. The method was sought validated for 92 pesticides in wheat, rice, rye, oat, 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

Samples and sample preparation: The samples are milled with a sieve at 1mm.

Extraction: The extraction procedure was performed with an acetate-buffered version of the QuEChERS method. The adopted method was previously validated by Herrmann et al. Five grams of sample was weighed into a 50 mL centrifuge tube; 10 mL of cold deionized water and ceramic homogenizers were added; and the tubes were shaken vigorously so that the sample was soaked thoroughly. Afterward, 10 mL of acetonitrile was added, and the tubes were shaken vigorously by hand for 1 min. A buffer-salt mixture from Thermo ScientificTM, consisting of 4 g magnesium sulfate (MgSO4), 1 g sodium chloride (NaCl), 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogencitrate sesquihydrate was added. The tubes were shaken with an automatic shaker (SPEX SamplePrep 2010 Geno/Grinder®) for 1 min at 750 rpm and then centrifuged for 10 min at 4500 rpm with a Heraeus™ Multifuge™ X3 Centrifuge.

Clean-up: After centrifugation, aliquots comprising 8 ml of the acetonitrile extracts were transferred to 15 mL centrifuge tubes and stored in a freezer for a minimum of 1 hour at -80ºC. The still-cold extracts were centrifuged in a cool centrifuge (at 5ºC) for 5 min to precipitate the low-soluble matrix co-extractives. Then 6 mL of acetonitrile extract was transferred to SupelTMQuE tubes containing 150 mg of PSA and 900 mg of MgSO4. The tubes were shaken in an automatic shaker for 1 min at 750 rpm and then centrifuged for 5 min at room temperature (20ºC) at 4500 rpm. Subsequently, 4 mL of the cleaned-up extracts was transferred into 15 ml centrifuge tubes, and 40 µl of 5% formic acid solution in acetonitrile was added to each extract to adjust the pH for storage stability. The extracts were later diluted by a factor of 2 with acetonitrile (0.25 g of sample/mL) to obtain the same matrix concentrations as those in the calibration standards, according to the in-house routine procedure for the quantitative methods.

Quantification and qualification: The final extracts are analysed by GC-Orbitrap-MS. The quantification ion and at least 2 qualifiers and the retention time are shown in **Appendix 1**.

GC-Orbitrap-MS: The system consists of a Trace 1300 Series GC, a TriPlus RSH Autosampler-GC liquids and an Exactive GC-Orbitrap-MS.

The pesticide residues were separated on a TG-5SILMS 30mx0.25mmx0.25µm column. The oven starting temperature was set at 60 °C for 1.5 min. The temperature was increased to 90 °C at a rate of 25°C/min and held for 1.5 min, then up to 180 °C at 25°C/min, then further after to 280°C at 5 °C/min, and finally up to 300°C at a rate of 10°C/min. This temperature was maintained for 12 min. The samples were injected in PTV mode and the injection volume was set to 1 µL.

The data was acquired by full scan mode covering a range of 50-500 m/z. The ion source operate through positive EI and the ion source temperature was set to 280 °C. The MS transfer line was set to 280°C. The Orbitrap operates at a resolution of 60 000 and the AGC target was set to 1e6.

3. Validation design

The method was sought validated for 92 pesticides. The validation was performed on 5 replicates of each matrix (wheat, rye, rice, oat, barley) at each of the three spiking levels; 0.005, 0.01 and 0.02 mg/kg. A blank sample of each commodity was included. Blanks of barley, wheat and rye were obtained from EUPT-C6, C8, C10 respectively. Oat and rice samples were bought from the market, milled freshly before the analysis, and analysed and screened for pesticides.

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at 6 calibration levels within the range of 0.333, 1, 3.33, 10, 33.3 and 100 ng/ml. The calibration curves were in generally 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.

5. Validation parameters

Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides on all three spiking levels (0.005 mg/kg, 0.01 mg/kg and 0.02 mg/kg). 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 (RSD_r) in this validation was calculated from the 25 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 (0.005 mg/kg, 0.01 mg/kg and 0.02 mg/kg) with the relevant pesticides.

Robustness

The QuEChERS method has, in connection with the development of the method, been shown to be robust by Anastassiades et al. 2003[3].

Limit of quantification, LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria was met.

The obtained results including recovery, RSD_r, uncertainty and limit of quantification (LOQ) are presented 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 ≤20%[4].
2. The average relative recovery must be between 70 and 120%[5]. However, lower recoveries are accepted if the repeatability is low.

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

The analytical result is by default corrected for bias/recovery and the combined uncertainty is then given by:

$$U_c = \sqrt{(RSD^2/n) + RSD^2}$$

Where RSD is the repeatability uncertainty (RSD_r).

7. Results and conclusions

Validation results are presented in **Appendix 2**. A total of 77 compounds were validated in wheat, rye, and oat. 76 compounds were validated in rice and barley.

Among 92 compounds, 13 compounds could not be validated at 0.02 mg/kg (the highest spiking level): 2-3-4-6-Tetrachlorophenol, 2-4-6-Trichlorophenol, allidochlor, aziprotryne, chinomethionate, dimethipin, fluoroglycofen-ethyl, imibenconazole, siduron, tetrahydrophthalimide_cis-1-2-3-6, thiocyclam-hydrogen-oxalate, trietazine and XMC. Therefore a LOQ could not be assigned. Among these compounds, 1 compound (thiocyclam-hydrogen-oxalate) was validated for screening but failed the quantitation validation with poor recoveries (<50%). Some compounds showed low sensitivity and therefore could not be validated at the spiking levels of 0.02 mg/kg and below: azipotryne, dibutyl chlorendate, fluroglycofen-ethyl, tiretazine, tetrahydrophthalimide_cis-1-2-3-6. Other compounds may not be successfully recovered with QuEChERS extraction due to the complexity of the matrix: 2-3-4-6-tetrachlorophenol and 2-4-6-trichlorophenol. Some compounds may be more suitable on LC e.g. azipotryne, siduron, trietazine and XMC.

Eventhough the method validation included the 5 different matrices, some pesticides showed a different behaviour with each matrix. Therefore, for butafenacil, a LOQ of 0.005 mg/kg was achieved for rye, rice and wheat, and a higher LOQ of 0.01 was achieved for oat and barley.
For butamifos, a LOQ of 0.005 mg/kg was achieved for all the matrices except for rice for which a higher LOQ of 0.01 mg/kg was demonstrated. For butylate, a LOQ of 0.005 mg/kg was obtained with the different cereal matrices except for oat (LOQ: 0.02 mg/kg).
The LOQ of pretitachlor was demonstrated at 0.005 mg/kg, except for barley (LOQ: 0.01 mg/kg).
2-4-D methyl ester was not validated with rice while a LOQ of 0.005 mg/kg was achieved with wheat, rye, oat, and barley.

More compounds should be validated for quantitation on the GC-Orbitrap-MS in the frame of transposing the quantitation methods from low to high Resolution Mass Spectrometry.

9. References

1 DIRECTIVE 2006/77/EC: Amending Annex I to COMMISSION Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for organochlorine compounds in animal feed.

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 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.

4 Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed, Document SANTE/11813/2017, 21–22 November 2017 rev.0, European Commission, Brussels, 2017.

Appendix 1. The quantification ion and at least 2 qualifiers and the retention time of the studied compounds.

Pesticides	Rt (min)	Target Peak	Confirming ion	Confirming ion	Confirming ion	Confirming ion
2-4-5-T-methylester	12.15	232.97668	208.93227	267.94580		
2-4-DB-methylester	12.98	161.96340	125.98667	97.99173	63.02294	
2-4-D-butylester	13.39	185.00000	219.96881	174.97125	161.96339	144.96057
2-4-D-methylester	10.59	199.01565	233.98468	174.97121	160.95560	132.96077
2-6-Dichlorobenzamide	10.95	172.95555	145.96840	188.97421		
4-4-Dichlorobenzophenone	15.07	138.99452	110.99969	215.02588	249.99478	
Allidochlor	7.91	138.09134	132.02086	56.04947	96.08073	124.07574
Ancymidol	18.46	228.08933	107.02408	121.06490	215.08098	135.04413
Anilofos	22.38	124.98206	183.99821	170.96977	154.04178	
Aramite	17.94	185.00330	175.11170	135.08050		
Aspon	14.44	210.90481	252.95190	294.99857	336.03824	
Atraton	11.51	196.11929	154.07248	169.09583	211.14281	
Atrazine-Desethyl	10.84	172.03845	145.01509	110.04623	187.06197	
Azaconazole	17.98	216.98176	86.03631	144.96063	174.95259	
Benodanil	19.29	230.93014	196.07578	202.93521	322.98016	
Benoxacor	12.92	120.04439	259.01630	176.07065	261.01343	
Benzoylprop-ethyl	21.3	105.03349	292.02911	77.03862	106.03694	
Butachlor	16.77	160.11208	176.10704	188.10704	276.19611	237.09154
Butafenacil	25.84	331.00918	123.99493	179.98473		
Butamifos	17	286.10251	258.07129	231.98286	200.01067	
Butylate	8.88	146.09980	156.13829	217.14949	174.09468	188.11038
Chinomethionate	16.63	205.99669	174.02461	173.01672	148.02161	116.04952
Chlorbenside	16.54	125.01525	127.01241	89.03864	267.98764	
Chlordimeform	10.85	181.05270	196.07622	152.02637	89.03864	
Chloroneb	9.48	190.96611	192.96306	205.98959	207.98662	
Chlorthiamid	13.28	169.98258	171.97961	204.95145	206.94856	
Chlorthion	15.06	124.98224	296.96225	109.00500	279.95984	
Crimidine	9.56	142.02923	156.03229	127.00585	120.05566	
Cycloate	10.65	154.12264	55.05426	83.08553	72.04443	
Cyfluthrin	26.5	206.06004	199.05540	127.03103	226.06627	163.00743
Dicamba-methylester	9.59	202.96611	187.94266	159.94789	96.98405	233.98433
Dicapthon	14.93	261.99336	124.98218	216.00027		
Dichlormid	8.35	172.05237	124.07581	136.07574	165.98206	
Dichlorprop-methylester	10.37	161.96337	188.98686	248.00027	190.98390	
Diclobutrazol	17.96	270.01954	158.97626	200.98694		
Diclofop-methyl	20.72	252.98176	254.97881	340.02637	281.01306	184.05188
Diphenamid	15.25	167.08553	165.06985	72.04443	152.06207	
Dithiopyr	13.86	286.04857	306.05460	258.05353	354.05789	
Etaconazole	18.75	245.01306	190.96609	172.95551	190.96609	172.95551

Pesticides	Rt (min)	Target Peak	Confirming ion	Confirming ion	Confirming ion	Confirming ion
Famphur	19.67	218.01611	93.01006	124.98219	217.00821	184.98216
Fenobucarb	10.32	121.06479	91.05429	150.10396	93.06996	
Fenoprop-methylester	11.81	195.92440	222.94795	187.97900	166.92162	
Fluchloralin	12.2	306.06962	326.01499	264.02267	248.02775	310.02008
Fluridone	27.85	328.09438	189.06989			
Hexazinone	20.36	171.08765	83.06037	71.06043	128.08188	
Imazamethabenz-methyl	17.06	144.04439	187.05019	214.07364	256.12085	
Iprobenfos	12.82	204.00045	123.02634	171.02054	246.04747	202.99272
Isazophos	12.47	118.98809	162.04285	161.03506	177.01221	
Isocarbamid	12.02	142.06110	130.06131	113.03457		
Isocarbophos	14.94	230.00353	121.02850	120.02070		
Lethane	9.63	86.00590	60.00282	89.00556	61.01050	
Mefenpyr-Diethyl	21.22	252.99299	271.00388	227.01382	299.03519	
Methabenzthiazuron	11.01	136.02155	164.04025	135.01381	163.03233	
Metolcarb	9.15	108.05697	107.04911	90.04642		
Mexacarbate	12.56	165.11482	164.10701	150.09137	222.13591	
MGK-264	15.25	164.07061	121.06485	111.03148	98.02366	209.14105
Nitrothal-isopropyl	15.04	194.00840	212.01895	236.05540	254.06598	
Norflurazon	19.9	303.03808	145.02602	173.03217	302.03027	
O-O-O-Triethylphosphorothioate	6.93	170.01611	93.00999	142.98926		
Pentanochlor	14.36	141.03398	197.06018	106.06521	239.10738	
Phenothrin	22.87	183.08044	123.11688	350.18765	168.05695	
Plifenate	13.45	169.96846	174.97122	216.98192	239.90622	
Pretilachlor	17.42	162.12773	262.18060	202.12267	238.09944	
Profluralin	11.93	318.06962	186.04010	248.02812	330.10599	232.03308
Prometryn	13.79	241.13557	226.11209	184.06514	199.08867	169.05424
Quizalofop-ethyl	27.15	372.08714	243.03227	244.04034	163.00581	
Rabenzazole	14.99	212.10565	170.07130	195.07912	118.05263	
Secbumeton	12.5	196.11929	169.09576	154.07243	210.13493	
Sulfallate	11.3	188.05622	88.02162	72.08081	59.99033	
SWEP	11.82	186.95860	218.98480	123.99497		
Tebupirimfos	12.74	186.95860	218.98480	123.99497		
Tebutam	11.11	190.12264	142.12264	233.17742		
Terbutryn	14.14	226.11209	185.07297	170.04947	241.13554	
Thenylchlor	20.51	288.10528	168.01149	141.03688	132.08095	127.02128
Thiazopyr	14.57	363.11264	306.00809	381.06897		
Thiocyclam-hydrogen-oxalate	9.53	135.01709	56.04951	71.07301		
Tiocarbazil	15.08	100.07569	156.13829	91.05428		
Tralkoxydim	23.51	137.04713	109.05244	283.15671		
Tribufos	17.69	201.97042	258.03302	146.91570	168.99049	
Triclopyr-methylester	11.16	209.92747	145.95590	179.91687		
Vernolate	9.01	128.10699	86.06010	161.08693	146.09993	

Appendix 2. Recovery, RSD_r, uncertainty and limit of quantification (LOQ)

	0.02 mg/kg				0.01 mg/kg				0.005 mg/kg				
	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	LOQ (mg/kg)
2-4-5-T-methylester	82	8	9	40	89	4	10	30	99	4	7	16	0.005
2-4-DB-methylester	81	7	7	41	86	3	12	37	94	5	19	41	0.005
2-4-D-butylester	86	7	7	31	92	2	6	20	101	2	5	11	0.005
2-4-D-methylester*	78	5	5	46	84	3	11	40	87	3	12	35	0.005
2-6-Dichlorobenzamide	83	7	8	38	85	2	10	35	92	2	10	26	0.005
4-4-Dichlorobenzophenone	83	7	8	37	90	2	6	24	102	2	6	13	0.005
Ancymidol	82	7	10	42	99	5	8	17	93	4	10	24	0.005
Anilofos	95	5	11	24	101	5	6	13	120	4	5	41	0.005
Aramite	99	7	11	23	110	6	11	30	125	5	10	54	0.02
Aspon	90	7	7	24	100	3	7	15	120	3	6	43	0.005
Atraton	86	8	8	32	87	6	10	34	91	5	11	29	0.005
Atrazine-Desethyl	82	7	8	40	83	3	10	39	90	3	11	30	0.005
Azaconazole	98	7	10	21	109	5	8	24	120	5	8	42	0.005
Benodanil	96	7	9	20	103	5	7	16	113	3	7	30	0.005
Benoxacor	93	8	14	31	90	3	6	24	100	4	8	15	0.005
Benzoylprop-ethyl	93	6	7	19	98	2	3	7	107	2	5	16	0.005

	0.02 mg/kg				0.01 mg/kg				0.005 mg/kg				
	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	LOQ (mg/kg)
Butachlor	95	6	6	16	104	3	5	13	120	3	4	41	0.005
Butafenacil*	100	6	17	35	112	6	16	39	122	2	7	47	0.005
Butamifos*	99	7	8	16	101	7	12	24	107	8	14	32	0.005
Butylate*	86	8	10	35	84	4	12	40	77	2	9	49	0.005
Chlorbenside	72	7	12	61	81	2	10	44	91	2	11	29	0.005
Chlordimeform	83	6	11	41	92	4	25	54	128	3	18	67	0.1
Chloroneb	87	9	9	33	83	6	10	40	80	7	13	48	0.005
Chlorthiamid	77	11	18	59	81	5	13	47	105	3	20	41	0.005
Chlorthion	71	7	9	62	86	5	12	37	114	3	9	33	0.005
Crimidine	84	7	8	35	83	4	12	43	83	4	11	42	0.005
Cycloate	85	7	8	35	87	8	21	50	70	14	13	67	0.01
Cyfluthrin	102	7	17	35	109	7	7	24	120	6	8	44	0.005
Dibutyl_chlorendate	83	8	8	38	85	11	11	37	116	7	8	35	0.005
Dicamba-methylester	88	8	9	29	86	9	14	40	81	5	14	47	0.005
Dicapthon	71	8	11	62	87	6	14	40	110	4	11	30	0.005
Dichlormid	91	7	9	26	84	13	19	49	80	7	10	45	0.005
Dichlorprop-methylester	86	7	8	32	88	4	11	32	89	3	11	31	0.005
Diclobutrazol	94	21	22	46	113	7	12	35	128	5	9	59	0.01

	0.02 mg/kg				0.01 mg/kg				0.005 mg/kg				
	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	LOQ (mg/kg)
Diclofop-methyl	92	8	8	23	101	3	5	9	114	4	6	30	0.005
Diphenamid	94	7	6	17	99	2	3	6	110	2	4	21	0.005
Dithiopyr	94	6	6	17	101	3	4	9	107	3	6	19	0.005
Etaconazole	85	17	18	47	103	5	5	12	129	3	4	59	0.01
Famphur	95	7	11	25	105	5	10	23	118	3	5	38	0.005
Fenobucarb	88	7	10	32	89	5	14	36	90	6	17	39	0.005
Fenoprop-methylester	86	8	9	33	90	4	9	28	96	3	8	19	0.005
Fluchloralin	62	8	17	83	82	6	17	50	98	6	15	31	0.005
Fluridone	94	6	18	38	97	5	10	21	112	14	15	39	0.005
Hexazinone	101	6	7	15	113	3	4	27					0.01
Imazamethabenz-methyl	89	9	14	36	88	6	10	31	92	6	13	30	0.005
Iprobenfos	90	6	7	24	94	3	8	20	105	2	7	18	0.005
Isazophos	95	6	6	16	102	4	6	13	118	2	6	38	0.005
Isocarbamid	81	7	7	41	86	3	9	33	98	3	10	20	0.005
Isocarbophos	85	6	6	33	94	5	11	26	112	4	8	29	0.005
Mefenpyr-Diethyl	95	7	9	21	105	4	6	16	125	2	3	51	0.005
Methabenzthiazuron	80	7	8	43	78	6	11	49	79	3	13	50	0.005
Mexacarbate	74	7	14	59	91	3	15	36	112	2	13	37	0.005

	0.02 mg/kg				0.01 mg/kg				0.005 mg/kg				
	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	LOQ (mg/kg)
MGK-264	97	6	6	14	108	4	5	19	136	6	7	74	0.01
Nitrothal-isopropyl	76	4	6	50	91	5	10	28	116	3	8	35	0.005
Norflurazon	94	7	8	21	102	4	4	10	120	3	3	41	0.005
O-O-O-Triethylphosphorothioate	104	13	15	31	0							200	0.02
Pentanochlor	91	6	6	23	95	3	6	15	106	3	7	18	0.005
Phenothrin	89	6	7	26	98	2	7	14	119	4	6	41	0.005
Plifenate	87	7	8	30	92	6	8	23	102	5	7	14	0.005
Pretilachlor*	99	8	11	23	111	8	14	35	115	4	5	32	0.005
Profluralin	78	7	12	49	85	6	13	41	91	7	11	29	0.005
Prometryn	95	6	7	16	102	3	5	11	120	2	5	42	0.005
Quizalofop-ethyl	96	7	19	40	101	7	11	22	120	7	10	45	0.005
Rabenzazole	91	7	6	22	99	2	4	9	111	2	5	24	0.005
Secbumeton	90	7	7	26	94	2	6	18	100	2	9	18	0.005
Sulfallate*	76	9	14	57	81	3	16	50	86	3	17	45	0.005
SWEP	83	7	7	36	86	4	9	33	99	4	9	18	0.005
Tebupirimfos	86	7	8	32	90	3	8	25	100	3	9	18	0.005
Tebutam	72	8	9	58	87	4	10	33	82	3	11	43	0.005
Terbutryn	90	7	7	25	95	4	6	16	105	2	6	16	0.005

	0.02 mg/kg				0.01 mg/kg				0.005 mg/kg				
	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	Recovery, %	RSDr, %	RSDR, %	Combined Expanded Uncertainty (%)	LOQ (mg/kg)
Thenylchlor	96	7	7	16	101	4	5	11	114	4	5	29	0.005
Thiazopyr	101	7	7	15	108	6	7	22	106	12	11	26	0.005
Tiocarbazil	86	7	8	33	92	3	7	21	100	4	9	19	0.005
Tralkoxydim	64	7	11	76	71	6	15	65	77	8	17	57	0.005
Tribufos	94	7	9	23	105	6	11	25	116	4	9	38	0.005
Triclopyr-methylester	84	7	8	36	87	3	9	33	93	3	10	25	0.005
Vernolate	79	9	13	49	78	4	17	56	71	4	18	68	0.02

*A LOQ of 0.005 mg/kg was achieved for butafenacil in rye, rice, and wheat and a LOQ of 0.01 mg/kg was achieved for butafenacil in oat and barley.

*A LOQ of 0.005 mg/kg was achieved for butamifos in all matrices except in rice (0.01 mg/kg).

*A LOQ of 0.005 mg/kg was achieved for butylate in all matrices except in oat (0.02 mg/kg).

*A LOQ of 0.005 mg/kg was achieved for pretitachlor in all matrices except in barley (0.01 mg/kg).

*2-4-D-methylester was not validated for rice.

*Sulfallate was not validated for barley