EURL for Cereals and Feeding stuff

National Food Institute

Technical University of Denmark

Screening Validation Report 10

Screening/quantitative methods for pesticide residues in cereals using Exactive™ GC Orbitrap™ GC-MS system from Thermo Fisher Scientific

Mette Erecius Poulsen

Elena Hakme

Ederina Ninga

January 2023

Contents

1 Introduction 3
2 Principle of analysis 3
GC conditions 3
Orbitrap conditions 4
Softwares 4
3 Validation plan 4
4 Database 5
5 Data Processing 5
6 Screening Results 6
7 Quantitative Results 6
8 Comparison between screening and quantitative approaches 6
9 Conclusions 7
10 References 7
Appendix 1. 8
Appendix 2. 11
Appendix3. 12
Appendix 4.. 17
Appendix 5. 18

1 Introduction

High Resolution Mass Spectrometry allows target screening processing methods involving a full scan MS acquisition method and screening against accurate mass database of more than 600 compounds covering a wide range of pesticides.

This report describes the screening and quantitative validation of the QuEChERS method combined with the Exactive™ GC Orbitrap™ GC-MS/MS from Thermo Fisher Scientific. The method was sought validated for 124 analytes in cereals at three screening detection limits (SDL) of 0.005, 0.01 and 0.02 mg/kg.

The method validated in this report is based on the QuEChERS extraction procedure for dry matrices (<30% water content) according to the document EN 15662:2008[1].

2 Principle of analysis

Extraction method

Cold water, acetonitrile and an internal standard are added to the milled sample and the sample is shaken. Salt and buffer mixture is added and the sample is shaken again. After centrifugation the supernatant is frozen at -80 ºC for one hour. After another centrifugation at 5 ºC the supernatant is transferred to a tube with PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract is obtained.

Different cereal samples were spiked at 0.005, 0.01 and 0.02 mg/kg with a mixture of pesticide standards and extracted by QuEChERS method. Final extracts were diluted 2-fold then analysed by GC-Orbitrap.

Instrumentation

GC conditions

GC-system: Trace 1300 series GC
Autosampler: TriPlus RSH Autosampler-GC Liquids.
Column: TG-5SILMS 30mx0.25mmx0.25µm.
Carrier gas: Helium
Carrier flow: 1.2 ml/min
Oven programme: The initial 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.
Injector: PTV
Injection volume: 1 µL.

Orbitrap conditions

Orbitrap instrument: Exactive GC-Orbitrap-MS
Ionisation mode: EI positive
Ion source temperature : 280 °C
MS transfer line temperature: 280 °C
Filament on delay: 6 min
Acquisition mode: Full MS

Properties of full MS

Resolution: 60 000
AGC target: 1e6
Scan rage: 50 to 500 m/z
Scan runtime: 6 to 32 min

Softwares

Software for tuning: Thermo Exactive GC
Software for instrument set up and method development: Thermo Scientific Xcalibur Instrument Setup
Software for data acquisition and processing: Thermo TraceFinder 5.1 General Quan

3 Validation plan

According to SANTE/11312/2021 the method should be validated on minimum 20 samples. The samples selected should represent multiple commodities from the same commodity group, with a minimum of two samples for each individual commodity included and will be representative for the intended scope of the laboratory. Five samples of five different types of cereal samples were spiked, which results in a total of 25 samples of each spiking level. Blanks of barley, oat, rice, rye and wheat were spiked with 4 standard mixtures called 1, 2, 7 and 8, containing 172 different compounds altogether. Not all the compounds included in the mix are GC amendable. The different cereal samples were spiked at 0.005, 0.01 and 0.02 mg/kg. All extracts were then analysed by GC-Orbitrap-MS. In total, 125 pesticides had been evaluated, 117 in screening qualitative mode and 111 in quantitative mode.

According to SANTE guidelines, the SDL of the qualitative screening method is the lowest level at which an analyte has been detected in at least 95% of the samples (i.e. an acceptable false-negative rate of 5%). This means that only 1 out of 20 spiked samples are allowed to be non-detected. For qualitative method recoveries should be between 70-120% and repeatability/reproducibility below 20%. Recoveries can be accepted outside 70-120% if repeatability/reproducibility is well below 20% and results are corrected for recoveries.

4 Database

The accurate mass Thermo database include 639 pesticides. Among these compounds, approximately 450 compounds were checked by injecting available standards, adding fragment ions and correcting for retention time. The remaining compounds were kept in the database to screen against without standard confirmation. Among the database are the 117 compounds for which screening validation was performed and presented in this report, while 111 was included in the quantitative method.

The database contains the compound name, theoretical exact masses for at least three fragment ions (at least one target ion and 2 confirming ions) and expected retention time information.

5 Data Processing

Data were processed using target screening experiment. The screening was performed using the TraceFinder software against the modified High Resolution Thermo database including 639 pesticides.

Compound detection and identification or screening processing parameters were based on

- peaks with threshold override of 50 and S/N of 5 and mass tolerance of 5 ppm
- retention time (window override: 60 s)
- fragment ions with a minimum of 2 fragment ions, and an intensity threshold of 500, mass tolerance of 5 ppm

As for peak detection parameters, the detection algorithm used is Genesis with Nearest Rt as detection mode.

If for reasons of peaks settings, some compounds could not be confirmed by automated Target screening, a parallel quantitation method was developed exporting the targeted compounds from the compound database to the Quantitation Method in the Thermo TraceFinder software, and therefore checked by comparing to the standards peaks and the MS requirements.

Data was also processed using TraceFinder quantitative method approach. For this approach calibration curves were included to calculate the amount of analyte in the spiked samples in order to calculate the recoveries and repeatability.

6 Screening Results

The validation results are listed in Appendix 1 together with the masses used for detection and confirmation. The masses listed in the tables are the positive exact masses of the fragments, e.i. the mass of the fragment minus the weight of one electron (0.0005 Da). Of the 117 compounds evaluated, 80 were validated. SDL of 0.005 mg/kg was obtained for 57 compounds, an SDL of 0.01 mg/kg was achieved for 10 compounds and an SDL of 0.02 mg/kg was achieved for 13 compounds. Some of the SDL did not cover oat.

Thirty-eight compounds were not validated and are listed in Appendix 2. In the Tracefinder Screening method it is not possible to choose the detection mode for individual compounds. E.g., it is only possible to select to search for either the highest peak or the nearest RT. By that some compounds will not be found. The processing method is very dependent on correct RT. Apart from that, some of the compounds may be more suitable on LC e.g. 2-3-5-Trimethacarb, 4-Nonylphenol, oxadiargyl and promecarb. Some compounds showed low sensitivity (did not fulfil the MS requirements for identification at low concentrations in the standards): fenuron, flumeturon, isoxaben. Other compounds are not successfully recovered with QuEChERS extraction: Captafol, clopyralid, cymoxanil, dazomet, diafenthiuron, flumioxazin.

7 Quantitative Results

The validation results are listed in Appendix 3. Of the 111 compounds evaluated, 96 were validated, 71 with a Limit Of Quantification, LOQ, of 0.005 mg/kg, 18 at 0.01 mg/ml and 7 at 0.02 mg/kg. All results can be seen in **Appendix 3**.

Fourteen compounds were not validated. Two of them were not GC amendable or the ions were not correct, because not calibration curve could be created. Poor or non recovery was observed for bioresmethrin, clopyralid, dazomet, dioxacarb, fenuron, fluometuron, metamitron, and tralomethrin. Diafenthiuron and isoxaben showed poor sensitivity while buturon/monuron did not fulfil requirements for precision. A list of not validated compound is shown in **Appendix 4**.

8 Comparison between screening and quantitative approaches

The two processing methods covered almost the same number of compounds, but the scope was not exactly the same. Eight compounds were included only in the quantitative method (7 were validated) and 14 only in the screening method (2 were validated). In general, the quantitative method validated more compound and at the lowest level.

Forty-two compounds were validated at 0.005 mg/ml level for both methods. An overview of the results can be seen in Table 1 and the full list of quantitative LOQs and screening SDLs and be

seen in **Appendix 5**. Both methods can be updated with compounds that were validated with the other method.

Table 1. Number of compounds validated at different quantitative LOQs and screening SDL or not validated or not in scope.

LOQ / SDL	0.005 mg/ml	0.01 mg/ml	0.02 mg/ml	Not validated	Not in scope	Total
0.005 mg/ml	42	5	4	4	2	57
0.01 mg/ml	8	1		1		10
0.02 mg/ml	8	4	1			13
Not validated	7	8	2	9	12	38
Not in scope	6			1		7
Total	71	18	7	15	14	125

9 Conclusions

Of the 125 compounds evaluated, 80 were validated in screening mode. SDL of 0.005 mg/kg was obtained for 53 compounds, an SDL of 0.01 was achieved for 10 compounds and an SDL of 0.02 was achieved for 13 compounds. 96 were validated in quantitative mode. LOQ of 0.005 mg/kg was obtained for 71 compounds, an LOQ of 0.01 mg/kg was achieved for 18 compounds and an LOQ of 0.02 mg/kg was achieved for 7 compounds.

The validated pesticides in screening mode fulfilled the following screening detection criteria, retention time override of 60s, mass accuracy of target peak < 5ppm, and mass accuracy of at least 2 fragment ions ≤ 5ppm. In quantitative mode most recoveries were between 70-120% and repeatability/reproducibility below 20%. Recoveries outside 70-120% were accepted for compounds where repeatability/reproducibility is well below 20% and results consequently can be corrected for recoveries.

10 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. Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No. SANTE/11312/2021.

Appendix 1. Screening detection limits (SDL) and the exact masses of target peaks and at least 2 confirming ions for each compound. The masses are imported from the high accurate mass database are the exact masses of M^+.

Pesticides	SDL (mg/kg)	Rt (min)	Target ion	Confirming ion	Confirming ion	Confirming ion	Confirming ion
2-phenylphenol	0.005	9.57	170.07262	115.05423	141.06988		
4-Nonylphenol	0.02	13.09	107.04914	220.18217	77.03858	108.0525	
Alachlor	0.005	13.42	160.11208	188.10699	146.09643	132.08078	202.12264
Allethrin	0.005	21.68	79.05423	81.06988	123.11683	136.08827	136.08827
Ametryn	0.005	13.49	170.04949	212.09644	227.11992	185.07297	
Aminocarb	0.005	11.81	151.09917	136.07569	120.05697	108.05697	
Bifenazate	0.005	21.81	196.07569	258.09989	152.06205	214.11006	300.14684
Bioresmethrin	0.01	20.84	128.06205	123.11683	143.08553	171.08044	
Bromacil	0.005	14.09	204.96072	161.9549	187.93417		
Butralin	0.005	14.96	266.11353	220.10805	224.06658	236.10297	277.14209
Buturon	0.005	6.77	152.99759	125.00268	154.99464		
Carfentrazone-ethyl	0.005	19.53	312.05905	310.01894	330.02517	340.09035	
Chlorbufam	0.02	11.63	127.01833	164.02615	171.00816	152.99759	223.03946
Chlorfenprop-methyl	0.005	10.13	165.01017	125.01525	196.02856	167.00722	
Clodinafop-propargyl	0.01	19.99	238.00656	266.03786	349.05117	222.01116	
Crufomate	0.005	14.92	182.07293	256.10971	169.04147	108.02089	276.05509
Cyanazine	0.005	14.56	198.0541	225.065	240.0885	212.0572	
Cyanofenphos	0.005	19.66	156.98715	141.00999	169.04129	185.01845	
Cyanophos	0.005	11.95	109.00491	124.98206	124.98206	243.01135	78.99434
Cyflufenamid	0.005	17.95	188.01179	240.03181	157.01339	223.02509	
Cyprazin	0.005	13.12	212.06975	170.0228	226.0854	184.03845	134.04612
DEET	0.005	9.97	190.12264	91.05423	119.04914	162.09134	
Demeton-S	0.01	11.39	88.03412	113.9535	114.96133	170.01611	
Desmetryn	0.005	13.48	213.10427	198.08079	171.05732	156.03384	141.02294
Dichlobenil	0.005	8.26	170.96371	172.96076	100.01818		
Dichlofenthion	0.005	13.07	222.93801	279.00061	161.96337	250.96948	
Difenoxuron	0.005	14.61	241.07335	198.05496	226.04987		
Dioxacarb	0.005	8.78	121.02841	165.05462	149.05971	166.06245	
Edifenphos	0.005	19.69	109.01065	172.98206	186.04977	310.02456	
EPTC	0.005	8.3	128.10699	132.08415	86.06004	160.07906	
Esprocarb	0.005	14.42	162.12773	91.05423	222.09471		
Ethalfluralin	0.005	10.55	276.05905	316.09035	248.02775	292.05397	
Ethoprophos	0.005	10.48	157.96196	96.95077	126.99772	200.00891	199.00109
Etofenprox	0.005	27.22	163.11174	135.08044	107.04914	183.08044	
Etridiazole	0.02	9	182.91812	210.94942	212.94647	139.9123	

Pesticides	SDL (mg/kg)	Rt (min)	Target ion	Confirming ion	Confirming ion	Confirming ion	Confirming ion
Fenchlorphos	0.005	13.68	284.93034	124.98207	269.90704		
Fenothiocarb	0.01	16.59	72.04439	160.07906	94.04132	88.03412	
Fenoxaprop-ethyl	0.01	24.46	288.0422	361.07115	261.01872		
Fenpiclonil	0.01	21.12	235.99026	237.98731	201.0214	174.0105	166.05255
Flamprop-isopropyl	0.005	18.52	105.03349	276.0586	156.00108	258.06916	
Flonicamid	0.005	10.08	174.01612	146.02121	172.02428		
Fluacrypyrim	0.005	19.03	145.0648	189.0546	204.0781		
Furalaxyl	0.005	15.88	95.01276	242.11756	152.07061	146.09643	269.10465
Furathiocarb	0.005	22.47	135.08044	163.07536	194.0396	325.13423	
Furilazole	0.005	11.54	219.99266	146.03672	221.98971	262.00323	
Isodrin	0.005	15.45	192.93731	194.93436	262.85642	264.85347	260.85936
Isoxathion	0.01	18.02	177.02429	130.02874	159.01372	285.02192	313.05322
Lenacil	0.005	19.85	153.06585	136.03931	110.06004		
Leptophos	0.01	22.82	171.0028	376.89761	374.90056	155.02564	138.97659
Mefenacet	0.005	23.19	192.01138	136.02155	120.08078	148.07569	
Mepronil	0.005	19.21	119.04914	91.05423	210.06753	269.14103	
Metolachlor	0.005	14.5	162.127726	146.096425	211.075843	238.099318	
Monuron	0.005	6.77	152.99759	125.00268	154.99464	90.03383	
Nitrofen	0.02	18.2	202.01799	282.97975	284.9768	162.02308	252.98176
Novaluron	0.01	8.44	142.00542	144.00247	114.0105	308.99858	310.99538
Orbencarb	0.005	14.05	222.09471	100.07569	125.01525		
Oxadiargyl	0.02	18.54	150.0105	184.97936	212.97427		
Oxadiazon	0.005	17.44	174.95862	258.03212	302.02195	344.0689	
Paraoxon-methyl	0.02	12.34	230.02129	200.0233	186.00765	247.02403	
Pebulate	0.005	8.3	128.10698	132.08415	161.08689		
Pindone	0.005	12.39	173.023321	146.036231	105.033491	230.093746	
Prometon	0.005	11.5	168.08799	210.13494	183.11146	225.15841	
Propazine	0.005	11.94	214.0854	172.03845	229.10887	187.06192	
Propetamphos	0.005	11.86	138.0137	193.97972	109.9824	222.03483	
Pyraflufen-ethyl	0.02	19.97	349.01975	412.0199	338.9898	288.99032	
Pyrifenox	0.01	16.44	262.00591	186.95862	200.02615		
Pyroquilon	0.005	12.16	173.08352	130.06513	172.07569	144.08078	
Quinoxyfen	0.01	19.81	237.05844	272.0273	161.00268	306.99615	
Santonin	0.02	17.77	173.09609	135.08044	203.10666	246.12505	
Sebuthylazine	0.02	12.64	200.06975	202.0668	172.03845	138.07742	229.10887
Simetryn	0.005	13.48	213.10427	155.03859	170.04949	198.08079	
Sulprofos	0.005	19.27	156.0062	140.02904	112.92792	322.02794	
Tebuthiuron	0.005	9.54	156.05954	171.08302	89.0168	129.03607	

Pesticides	SDL (mg/kg)	Rt (min)	Target ion	Confirming ion	Confirming ion	Confirming ion	Confirming ion
Terbacil	0.005	12.36	161.01123	160.00341	116.99759	163.00828	
Terbumeton	0.005	11.73	169.09581	210.13494	154.07234	225.15841	
Terbuthylazine	0.005	11.94	214.0854	173.04627	138.07742	216.08245	229.10887
Thiobencarb	0.02	14.48	100.07569	125.01525	257.06357		
Trichloronate	0.005	14.96	108.98715	268.93542	296.96672	195.92422	
Triflumizole	0.02	16.04	205.99789	178.98699	278.0554	218.04795	287.04316
Trinexapac-ethyl	0.02	14	224.06793	95.04914	151.03897	137.02332	

Appendix 2. Not validated pesticides and ions used for detection.

Compounds	Target ion	Confirming ion	Confirming ion	Confirming ion	Confirming ion
2-3-5-Trimethacarb	121.0648	136.0883	91.05423		
2-4-Dimethylphenylformamide	120.0808	77.03858	149.0835	106.0651	
Anthraquinone	152.0621	208.0519	180.057	151.0542	
Atrazine-Desisopropyl	173.0463	158.0228	145.015	110.0461	
Bixafen	159.0365	413.0304	415.0275		
Bromophos-methyl	330.8778	328.8798	124.9822	332.8749	78.99452
Captafol	79.05423	79.05423	80.06205		
Chlorbromuron	123.9949	203.921	230.9081	291.9609	
Chlorthal-dimethyl	300.8801	298.8831	302.8772	331.8985	329.9015
Chlozolinate	186.9586	188.9557	185.9872	258.9798	260.9768
Clopyralid	146.9637	111.9949	76.01817	84.98395	
Coumaphos	225.985	96.95076	109.0049	362.0139	210.0078
Cycluron	127.0866	169.1335	198.1727		
Dazomet	89.02937	162.028	75.94359	71.99025	72.99807
Diafenthiuron	311.1338	296.1104	262.1226	254.0634	
Dimethachlor	134.0964	197.0602	148.0757	132.0808	
Dimethirimol	166.0975	96.04439	209.1523		
Dinoseb	211.0349	147.0315	163.0264	205.0608	193.0244
Ethirimol	209.1523	96.04439	170.0812		
Etoxazole	141.0147	187.1117	300.1195	330.13	
Fenuron	72.04439	164.0944	118.0651	147.0679	
Flumioxazin	354.101	259.0514	287.0827	326.1061	
Fluometuron	72.04439	159.029	187.024	232.0818	
Forchlorfenuron	119.0366	128.0136	153.9928	247.0507	
Haloxyfop	298.0816	298.0816	375.048	226.0474	179.9822
Hexaflumuron	201.9457	203.9428	302.9472	175.9479	304.9442
Isoxaben	165.0546	150.0312	107.0128		
Metamitron	104.0495	174.09	173.0822	202.0849	
Methoprene	81.06988	109.1012	91.05423	107.0855	111.0441
Metoxuron	228.066	183.0082			
Naled	78.99434	109.0049	144.9816	184.9765	
Nicotine	84.08078	133.076	161.1073	119.0604	162.1152
Permethrin	183.0804	127.0309	163.0076	168.057	
Pentachlorophenol	263.8465	265.8435	267.8406	269.8376	229.8854
Promecarb	135.0804	150.1039	107.0855		
Rotenone	192.1509	191.143	193.1587		
Temephos	465.9892	124.9821	108.9872		
Thionazin	143.0005	107.0604	96.9508	248.0379	

Appendix3. Validated compounds in quantitative mode.

Recoveries, repeatability (RSDr), internal reproducibility (RSDR), expanded uncertainty (U) and Limit of Quantification (LOQ) for pesticides validated on four commodities, barley, rice, rye and wheat using QuEChERS.

Compounds	*Spike level 0.002 mg/kg*					*Spike level 0.005 mg/kg*					Spike level 0.01 mg/kg					LOQ
	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	
2-3-5-Trimethacarb											67	4	19	77	19	0.02
2-4-Dimethylphenylformamide	131	2	13	66	13	89	10	17	41	17	64	3	11	75	11	0.005
2-phenylphenol	45	6	20	118	20	55	12	25	104	26	56	3	21	98	22	0.005
4-Nonylphenol											52	2	14	100	15	0.02
Alachlor	104	3	15	31	15	91	8	11	29	11	84	3	6	35	6	0.005
Ametryn	98	3	10	21	10	85	8	11	38	12	77	3	7	49	7	0.005
Aminocarb	95	2	12	26	12	76	8	11	54	12	67	3	9	68	10	0.005
Anthraquinone						99	19	24	49	24	76	4	7	49	7	0.005
Atrazine-Desisopropyl	102	3	11	24	12	88	7	11	32	11	78	4	8	47	8	0.005
Bifenazate	79	4	18	55	18	66	8	19	79	20	58	3	15	89	16	0.005
Bixafen						78	11	16	56	17	73	2	6	56	6	0.01
Bromacil	82	3	15	48	15	82	10	14	46	14	73	3	5	54	5	0.005
Bromophos-methyl	107	2	7	21	7	88	9	13	36	13	75	3	7	52	8	0.005
Butralin	114	2	6	32	6	85	7	11	37	11	71	4	7	59	7	0.005
Carfentrazone-ethyl	100	3	12	24	12	93	9	11	27	12	82	3	5	38	5	0.005
Chlorbromuron						102	6	8	17	8	73	17	16	62	16	0.005
Chlorbufam	98	4	10	20	10	83	11	15	46	15	74	3	7	55	7	0.005

Compounds	*Spike level 0.002 mg/kg*					*Spike level 0.005 mg/kg*					Spike level 0.01 mg/kg					LOQ
	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	
Chlorfenprop-methyl	83	3	17	48	17	68	6	9	66	10	68	3	6	65	6	0.005
Chlorthal-dimethyl	93	2	9	22	9	84	8	11	39	11	76	3	7	50	7	0.005
Chlozolinate	91	4	7	23	7	92	7	12	28	12	81	3	5	38	5	0.005
Clodinafop-propargyl	100	4	12	25	12	81	10	14	47	15	70	3	5	62	5	0.005
Coumaphos	85	10	12	39	12	78	14	19	58	19	67	4	9	68	9	0.005
Crufomate											60	4	5	80	6	0.005
Cyanazine	123	10	11	51	11	88	8	12	33	12	81	3	8	40	8	0.02
Cyanofenphos	92	3	14	32	14	84	10	12	41	13	76	3	5	50	5	0.005
Cyanophos	133	2	6	67	7	98	8	10	21	10	78	2	6	47	7	0.005
Cycluron	132	4	6	65	6	104	10	12	25	12	83	4	6	35	6	0.005
Cyflufenamid	133	3	3	67	3	101	7	8	16	8	81	3	5	40	5	0.005
Cyprazin	93	3	14	31	14	81	9	12	45	13	74	3	5	54	5	0.005
DEET	89	2	16	38	16	82	7	10	41	11	77	3	6	48	6	0.005
Demeton-S	100	3	9	19	9	86	6	11	35	11	78	3	9	47	9	0.005
Desmetryn	83	4	18	50	19	76	9	12	54	13	71	3	6	59	6	0.005
Dichlobenil	87	3	16	42	17	81	5	9	43	9	82	3	6	39	7	0.005
Dichlofenthion	87	3	15	41	16	74	8	12	57	12	72	3	7	57	7	0.005
Difenoxuron	111	3	12	34	13	83	9	12	42	13	71	4	6	59	7	0.005
Dimethachlor	92	3	15	36	16	85	9	12	39	12	78	3	6	45	6	0.005
Dimethirimol						58	8	16	90	16	58	3	11	87	11	0.005
Edifenphos	113	6	6	29	7	99	11	13	28	14	82	4	5	37	5	0.01
EPTC						55	7	19	99	19	73	4	10	58	10	0.005

Compounds	*Spike level 0.002 mg/kg*					*Spike level 0.005 mg/kg*					Spike level 0.01 mg/kg					LOQ
	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	
Esprocarb	81	3	17	51	17	74	9	13	58	14	71	4	8	60	8	0.01
Ethalfluralin	96	4	7	17	7	77	8	11	51	11	71	5	6	60	6	0.005
Ethoprophos	75	3	21	66	22	73	9	13	60	13	72	3	7	59	7	0.005
Etofenprox											59	3	5	83	5	0.005
Etoxazole	94	3	10	25	11	84	10	12	40	12	75	3	3	50	3	0.02
Etridiazole						111	7	9	28	9	89	3	4	24	4	0.005
Fenchlorphos	103	2	7	16	8	87	7	11	35	11	77	3	5	47	5	0.005
Fenothiocarb						60	16	18	89	19	62	4	9	77	9	0.005
Fenoxaprop-ethyl	91	4	11	29	11	79	9	13	50	14	70	4	6	61	6	0.01
Fenpiclonil	97	4	12	24	12	80	9	15	51	16	68	3	7	65	7	0.005
Flamprop-isopropyl						54	15	22	102	23	62	4	7	78	8	0.005
Flonicamid	102	4	14	29	14	87	11	17	42	17	74	4	8	55	8	0.01
Fluacrypyrim											43	5	10	116	10	0.005
Flumioxazin						74	9	14	60	15	61	11	18	86	19	0.02
Furalaxyl	84	4	13	41	13	84	10	14	42	14	79	3	7	45	7	0.01
Furathiocarb											81	4	12	46	13	0.005
Furilazole	108	3	12	30	12	94	7	10	24	10	82	3	6	39	6	0.02
Hexaflumuron						148	3	3	96	3	110	2	7	25	7	0.005
Isodrin	93	2	8	22	8	79	5	13	50	13	74	2	6	53	6	0.01
Isopropalin	102	2	11	22	11	74	8	13	58	14	66	4	8	70	8	0.005
Isoxathion						109	9	12	30	12	74	3	3	52	3	0.005
Lenacil	79	3	17	55	17	77	9	12	52	12	68	3	4	64	4	0.005

Compounds	*Spike level 0.002 mg/kg*					*Spike level 0.005 mg/kg*					Spike level 0.01 mg/kg					LOQ
	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	
Leptophos	92	3	11	27	11	75	9	16	60	17	70	3	6	60	6	0.005
Mefenacet	88	3	13	35	13	80	11	17	54	18	69	3	8	65	9	0.005
Mepronil	78	4	20	59	20	76	11	14	56	14	70	4	5	60	5	0.005
Naled	119	3	6	40	7	86	5	8	32	9	76	3	7	51	7	0.005
Nicotine											16	4	14	170	14	0.005
Nitrofen	108	3	8	23	8	82	9	13	45	14	67	4	4	66	4	0.02
Novaluron	87	7	17	43	17	79	12	13	51	14	71	5	16	66	16	0.005
Orbencarb	83	3	19	52	19	73	8	12	60	13	81	15	20	56	21	0.005
Oxadiargyl	89	5	11	31	11	71	10	14	65	14	64	3	3	72	3	0.005
Oxadiazon	86	4	7	31	7	75	11	13	57	13	69	3	6	62	6	0.005
Paraoxon-methyl	103	6	7	15	7	77	15	18	59	19	59	4	8	84	8	0.005
Pebulate						55	7	19	99	19	73	4	10	58	10	0.005
Permethrin						116	18	17	48	18	90	20	21	47	21	0.01
Promecarb						83	14	20	54	21	73	5	9	58	10	0.01
Prometon						65	12	16	76	16	69	11	17	71	18	
Propazine	91	3	12	30	12	83	8	11	40	11	75	3	6	51	6	0.01
Propetamphos	95	2	12	27	13	88	7	12	33	12	81	2	6	41	6	0.01
Pyraflufen-ethyl						63	15	20	85	21	62	5	10	79	11	0.005
Pyrifenox	83	5	13	43	13	79	9	13	50	13	72	4	8	58	8	0.005
Pyroquilon	82	3	14	46	14	80	10	14	49	14	74	4	7	54	7	0.01
Quinoxyfen	70	5	17	70	17	61	12	16	85	16	60	4	8	81	8	0.005
Santonin						88	9	22	52	23	74	4	10	55	10	0.005

Compounds	*Spike level 0.002 mg/kg*					*Spike level 0.005 mg/kg*					Spike level 0.01 mg/kg					LOQ
	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	
Sebuthylazine	77	4	16	56	16	78	10	14	53	14	75	3	6	52	6	0.005
Simetryn	80	4	18	55	19	76	9	12	55	12	72	3	6	57	6	0.01
Sulprofos	84	2	16	45	16	71	6	13	63	13	68	3	7	65	7	0.005
Tebuthiuron	60	5	22	91	22	65	12	17	79	17	67	3	7	68	7	0.005
Terbacil	83	3	16	47	16	78	11	15	54	16	71	3	7	60	7	0.005
Terbumeton	76	4	19	61	19	72	9	15	63	15	70	4	9	63	9	0.005
Terbuthylazine	92	3	12	29	12	84	8	11	40	11	75	3	6	51	6	0.005
Tetramethrin	84	5	13	43	14	80	7	12	46	12	72	4	4	57	4	0.005
Thiobencarb	64	4	21	85	22	66	10	13	73	14	65	3	7	71	7	0.005
Trichloronate	79	3	12	49	12	68	9	14	70	15	67	3	7	67	7	0.005
Triclopyr-methylester	96	3	13	27	13	86	6	9	34	9	78	2	4	45	4	
Triflumizole	92	7	18	41	19	86	9	19	48	20	74	4	16	63	17	0.005
Trinexapac-ethyl	98	5	14	28	14	52	5	9	99	9	30	7	15	144	16	0.005

Appendix 4. Not validated compound in quantitative mode.

Allethrin

Bioresmethrin

Buturon

Captafol

Clopyralid

Dazomet

Diafenthiuron

Dioxacarb

Fenuron

Fluometuron

Isoxaben

Metamitron

Methoprene

Monuron

Tralomethrin

Appendix 5. Obtained Screening Detection limits, SDL and Limit Of Quantification, LOQ

Compounds	SDL, mg/kg	LOQ, mg/kg
2-3-5-Trimethacarb	Not Validated	0.02
2-4-Dimethylphenylformamide		0.005
2-phenylphenol	0.005	0.005
4-Nonylphenol	0.02	0.02
Alachlor	0.005	0.005
Allethrin	0.005	Not validated
Ametryn	0.005	0.005
Aminocarb	0.005	0.005
Anilazine	Not Validated	
Anthraquinone	Not Validated	0.01
Atrazine-Desisopropyl		0.005
Bifenazate	0.005	0.005
Bifenox	Not Validated	
Bioresmethrin	0.01	Not validated
Bixafen	Not Validated	0.01
Bromacil	0.005	0.005
Bromophos-methyl	Not Validated	0.005
Butralin	0.005	0.005
Buturon	0.005	Not validated
Captafol	Not Validated	Not validated
Carfentrazone-ethyl	0.005	0.005
Chlorbromuron	Not Validated	0.01
Chlorbufam	0.02	0.005
Chlorfenprop-methyl	0.005	0.005
Chlorthal-dimethyl		0.005
Chlozolinate	Not Validated	0.005
Clodinafop-propargyl	0.01	0.005
Clopyralid	Not Validated	Not validated
Coumaphos	Not Validated	0.005
Crufomate	0.005	0.02
Cyanazine	0.005	0.005

Cyanofenphos	0.005	0.005
Cyanophos	0.005	0.005
Cycluron	Not Validated	0.005
Cyflufenamid	0.005	0.01
Cymoxanil	Not Validated	
Cyprazin	0.005	0.005
Dazomet	Not Validated	Not validated
DEET	0.005	0.005
Demeton-S	0.01	0.005
Desmetryn	0.005	0.005
Diafenthiuron	Not Validated	Not validated
Dichlobenil	0.005	0.005
Dichlofenthion	0.005	0.005
Difenoxuron	0.005	0.005
Dimethachlor	Not Validated	0.005
Dimethirimol	Not Validated	0.01
Dinoseb	Not Validated	
Dioxacarb	0.005	Not validated
Edifenphos	0.005	0.005
EPTC	0.005	0.01
Esprocarb	0.005	0.005
Ethalfluralin	0.005	0.005
Ethirimol	Not Validated	
Ethoprophos	0.005	0.005
Etofenprox	0.005	0.02
Etoxazole	Not Validated	0.005
Etridiazole	0.02	0.01
Fenchlorphos	0.005	0.005
Fenothiocarb	0.02	0.01
Fenoxaprop-ethyl	0.01	0.005
Fenpiclonil	0.01	0.005
Fenuron	Not Validated	Not validated
Flamprop-isopropyl	0.005	0.01
Flonicamid	0.005	0.005

Fluacrypyrim	0.005	0.02
Flumioxazin	Not Validated	0.01
Fluometuron	Not Validated	Not validated
Forchlorfenuron	Not Validated	
Furalaxyl	0.005	0.005
Furathiocarb	0.005	0.02
Furilazole	0.005	0.005
Haloxyfop	Not Validated	
Hexaflumuron	Not Validated	0.01
Isodrin	0.005	0.005
Isopropalin		0.005
Isoxaben	Not Validated	Not validated
Isoxathion	0.01	0.01
Lenacil	0.005	0.005
Leptophos	0.01	0.005
Mefenacet	0.005	0.005
Mepronil	0.005	0.005
Metamitron	Not Validated	Not validated
Methoprene	Not Validated	Not validated
Metolachlor	0.005	
Metoxuron	Not Validated	
Monuron	0.005	Not validated
Naled	Not Validated	0.005
Nicotine	Not Validated	0.02
Nitrofen	0.02	0.005
Novaluron	0.01	0.005
Orbencarb	0.005	0.005
Oxadiargyl	0.02	0.005
Oxadiazon	0.005	0.005
Paraoxon-methyl	0.02	0.005
Pebulate	0.005	0.01
Pentachlorophenol	Not Validated	
Permethrin I	Not Validated	0.01
Pindone	0.005	

Promecarb	Not Validated	0.01
Prometon	0.005	0.01
Propazine	0.005	0.005
Propetamphos	0.005	0.005
Pyraflufen-ethyl	0.02	0.01
Pyrifenox	0.01	0.005
Pyroquilon	0.005	0.005
Quinoxyfen	0.01	0.005
Rotenone	Not Validated	
Santonin	0.02	0.01
Sebuthylazine	0.02	0.005
Simetryn	0.005	0.005
Sulprofos	0.005	0.005
Tebuthiuron	0.005	0.005
Temephos	Not Validated	
Terbacil	0.005	0.005
Terbumeton	0.005	0.005
Terbuthylazine	0.005	0.005
Tetramethrin		0.005
Thiobencarb	0.02	0.005
Thionazin	Not Validated	
Tralomethrin		Not validated
Trichloronate	0.005	0.005
Triclopyr-methylester		0.005
Triflumizole	0.02	0.005
Trinexapac-ethyl	0.02	0.005