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National Food Institute

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Screening Validation Report 8

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

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Contents

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 500 compounds covering a wide range of pesticides.

This report describes the screening validation of the QuEChERS method combined with the Exactive™ GC Orbitrap™ GC-MS/MS from Thermo Fisher Scientific. The method was sought validated for 92 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

1.1. Extraction method

Cold water/ice 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 MgSO4. 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.

1.2. 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 : 280 °C
Filament on delay: 6 min
Acquisition mode: Full MS

<u>Properties of full MS</u>

Resolution: 60 000
AGC target: 1e6
Scan rage: 50 to 500 m/z
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 4.1 General Quan

3 Validation plan

According to SANTE/11813/20172 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. The samples were barley, rice, rye, oat and wheat. 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. The samples were spiked with 4 standard mixtures called 3, 4, 5 and 6, containing 147 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, 92 pesticides had been evaluated.

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 25 spiked samples are allowed to be non-detected.

4 Database

The accurate mass Thermo database include 639 pesticides. Among these compounds, 400 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 92 compounds for which screening validation was performed and presented in this report.

The database contains the compound name, theoretical exact masses for at least three fragment ions (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.

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 92 compounds evaluated, 81 were validated. SDL of 0.005 mg/kg was obtained for 54 compounds, an SDL of 0.01 mg/kg was achieved for 17 compounds and an SDL of 0.02 mg/kg was achieved for 10 compounds.

Twelve compounds were not validated and are listed in Appendix 2. An SDL > 0.02 could be assigned for two compounds (fluoroglycofen-ethyl and imibenconazole) if comparing with previous screening reports (S6) on GC-ToF where a value of 0.1 mg/kg was assigned for these 2 compounds and seen that the highest spiking level in this study was 0.02 mg/kg. Other compounds may be more suitable on LC e.g. azipotryne, siduron, trietazine and XMC. Some compounds showed low sensitivity (did not fulfil the MS requirements for identification at low concentrations in the standards): azipotryne, dibutyl chlorendate, fluroglycofen-ethyl, tiretazine, tetrahydrophthalimide_cis-1-2-3-6. Other compounds may not be successfully recovered with QuEChERS extraction: 2-3-4-6-tetrachlorophenol and 2-4-6-trichlorophenol.

7 Conclusions

Of the 92 compounds evaluated, 81 were validated. SDL of 0.005 mg/kg was obtained for 54 compounds, an SDL of 0.01 was achieved for 17 compounds and an SDL of 0.02 was achieved for 10 compounds.

The validated pesticides 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.

8 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/11813/2017.

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 Peak	Confirming ion	Confirming ion	Confirming ion	Confirming ion
2-4-5-T-methylester	0.010	12.15	232.97668	208.93227	267.94580		
2-4-DB-methylester	0.010	12.98	161.96340	125.98667	97.99173	63.02294	
2-4-D-butylester	0.005	13.39	185.00000	219.96881	174.97125	161.96339	144.96057
2-4-D-methylester	0.005	10.59	199.01565	233.98468	174.97121	160.95560	132.96077
2-6-Dichlorobenzamide	0.005	10.95	172.95555	145.96840	188.97421		
4-4-Dichlorobenzophenone	0.005	15.07	138.99452	110.99969	215.02588	249.99478	
Allidochlor	0.020	7.91	138.09134	132.02086	56.04947	96.08073	124.07574
Ancymidol	0.005	18.46	228.08933	107.02408	121.06490	215.08098	135.04413
Anilofos	0.005	22.38	124.98206	183.99821	170.96977	154.04178	
Aramite	0.020	17.94	185.00330	175.11170	135.08050		
Aspon	0.005	14.44	210.90481	252.95190	294.99857	336.03824	
Atraton	0.005	11.51	196.11929	154.07248	169.09583	211.14281	
Atrazine-Desethyl	0.005	10.84	172.03845	145.01509	110.04623	187.06197	
Azaconazole	0.005	17.98	216.98176	86.03631	144.96063	174.95259	
Benodanil	0.005	19.29	230.93014	196.07578	202.93521	322.98016	
Benoxacor	0.005	12.92	120.04439	259.01630	176.07065	261.01343	
Benzoylprop-ethyl	0.005	21.3	105.03349	292.02911	77.03862	106.03694	
Butachlor	0.005	16.77	160.11208	176.10704	188.10704	276.19611	237.09154
Butafenacil	0.005	25.84	331.00918	123.99493	179.98473		
Butamifos	0.010	17	286.10251	258.07129	231.98286	200.01067	
Butylate	0.005	8.88	146.09980	156.13829	217.14949	174.09468	188.11038
Chinomethionate	0.005	16.63	205.99669	174.02461	173.01672	148.02161	116.04952
Chlorbenside	0.005	16.54	125.01525	127.01241	89.03864	267.98764	

Pesticides	SDL (mg/kg)	Rt (min)	Target Peak	Confirming ion	Confirming ion	Confirming ion	Confirming ion
Chlordimeform	0.020	10.85	181.05270	196.07622	152.02637	89.03864	
Chloroneb	0.005	9.48	190.96611	192.96306	205.98959	207.98662	
Chlorthiamid	0.020	13.28	169.98258	171.97961	204.95145	206.94856	
Chlorthion	0.010	15.06	124.98224	296.96225	109.00500	279.95984	
Crimidine	0.005	9.56	142.02923	156.03229	127.00585	120.05566	
Cycloate	0.010	10.65	154.12264	55.05426	83.08553	72.04443	
Cyfluthrin	0.010	26.5	206.06004	199.05540	127.03103	226.06627	163.00743
Dicamba-methylester	0.005	9.59	202.96611	187.94266	159.94789	96.98405	233.98433
Dicapthon	0.005	14.93	261.99336	124.98218	216.00027		
Dichlormid	0.010	8.35	172.05237	124.07581	136.07574	165.98206	
Dichlorprop-methylester	0.005	10.37	161.96337	188.98686	248.00027	190.98390	
Diclobutrazol	0.005	17.96	270.01954	158.97626	200.98694		
Diclofop-methyl	0.005	20.72	252.98176	254.97881	340.02637	281.01306	184.05188
Diphenamid	0.005	15.25	167.08553	165.06985	72.04443	152.06207	
Dithiopyr	0.005	13.86	286.04857	306.05460	258.05353	354.05789	
Etaconazole	0.010	18.75	245.01306	190.96609	172.95551	190.96609	172.95551
Famphur	0.005	19.67	218.01611	93.01006	124.98219	217.00821	184.98216
Fenobucarb	0.005	10.32	121.06479	91.05429	150.10396	93.06996	
Fenoprop-methylester	0.010	11.81	195.92440	222.94795	187.97900	166.92162	
Fluchloralin	0.005	12.2	306.06962	326.01499	264.02267	248.02775	310.02008
Fluridone	0.020	27.85	328.09438	189.06989			
Hexazinone	0.010	20.36	171.08765	83.06037	71.06043	128.08188	
Imazamethabenz-methyl	0.010	17.06	144.04439	187.05019	214.07364	256.12085	
Iprobenfos	0.005	12.82	204.00045	123.02634	171.02054	246.04747	202.99272
Isazophos	0.005	12.47	118.98809	162.04285	161.03506	177.01221	
Isocarbamid	0.010	12.02	142.06110	130.06131	113.03457		
Isocarbophos	0.005	14.94	230.00353	121.02850	120.02070		
Lethane	0.020	9.63	86.00590	60.00282	89.00556	61.01050	
Mefenpyr-Diethyl	0.005	21.22	252.99299	271.00388	227.01382	299.03519	
Methabenzthiazuron	0.005	11.01	136.02155	164.04025	135.01381	163.03233	
Metolcarb	0.020	9.15	108.05697	107.04911	90.04642		
Mexacarbate	0.005	12.56	165.11482	164.10701	150.09137	222.13591	

Pesticides	SDL (mg/kg)	Rt (min)	Target Peak	Confirming ion	Confirming ion	Confirming ion	Confirming ion
MGK-264	0.005	15.25	164.07061	121.06485	111.03148	98.02366	209.14105
Nitrothal-isopropyl	0.005	15.04	194.00840	212.01895	236.05540	254.06598	
Norflurazon	0.005	19.9	303.03808	145.02602	173.03217	302.03027	
O-O-O-Triethylphosphorothioate	0.020	6.93	170.01611	93.00999	142.98926		
Pentanochlor	0.005	14.36	141.03398	197.06018	106.06521	239.10738	
Phenothrin	0.010	22.87	183.08044	123.11688	350.18765	168.05695	
Plifenate	0.010	13.45	169.96846	174.97122	216.98192	239.90622	
Pretilachlor	0.005	17.42	162.12773	262.18060	202.12267	238.09944	
Profluralin	0.020	11.93	318.06962	186.04010	248.02812	330.10599	232.03308
Prometryn	0.005	13.79	241.13557	226.11209	184.06514	199.08867	169.05424
Quizalofop-ethyl	0.010	27.15	372.08714	243.03227	244.04034	163.00581	
Rabenzazole	0.005	14.99	212.10565	170.07130	195.07912	118.05263	
Secbumeton	0.005	12.5	196.11929	169.09576	154.07243	210.13493	
Sulfallate	0.010	11.3	188.05622	88.02162	72.08081	59.99033	
SWEP	0.005	11.82	186.95860	218.98480	123.99497		
Tebupirimfos	0.005	12.74	186.95860	218.98480	123.99497		
Tebutam	0.005	11.11	190.12264	142.12264	233.17742		
Terbutryn	0.005	14.14	226.11209	185.07297	170.04947	241.13554	
Thenylchlor	0.005	20.51	288.10528	168.01149	141.03688	132.08095	127.02128
Thiazopyr	0.020	14.57	363.11264	306.00809	381.06897		
Thiocyclam-hydrogen-oxalate	0.005	9.53	135.01709	56.04951	71.07301		
Tiocarbazil	0.005	15.08	100.07569	156.13829	91.05428		
Tralkoxydim	0.010	23.51	137.04713	109.05244	283.15671		
Tribufos	0.005	17.69	201.97042	258.03302	146.91570	168.99049	
Triclopyr-methylester	0.005	11.16	209.92747	145.95590	179.91687		
Vernolate	0.005	9.01	128.10699	86.06010	161.08693	146.09993	

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

Pesticides	Target Peak	Confirming ion	Confirming ion	Confirming ion	Confirming ion
2-3-4-6-Tetrachlorophenol	229.88543	233.87949	231.88268	193.90875	
2-4-6-Trichlorophenol	195.92440	159.94800	131.95290		
Aziprotryne	182.04949	139.09782	196.06514		
Dibutyl_chlorendate	234.84372	368.82079	314.87800	260.85937	385.82353
Dimethipin	123.96473	118.00830	75.99774	108.97764	
Fluoroglycofen-ethyl	343.99320	315.00303	345.00102	301.01119	222.97682
Imibenconazole	375.02325	253.00423	170.96371	125.01525	
Siduron	93.05730	232.15701			
Tetrahydrophthalimide_cis-1-2-3-6	151.06278	123.06782	122.06023		
Trietazine	200.06970	186.05410	214.08540	229.10890	
XMC	122.07262	107.04914	121.02841	79.05423	