EURL for Cereals and Feeding Stuff

National Food Institute

Technical University of Denmark

Screening Validation Report 7

Screening method for pesticide residues in cereals using GC-QTOF from Agilent

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December 2017

Content

1 Introduction

Qualitative multi residue methods, especially those involving automated MS-based detection, offers laboratories a cost-effective means to extend their analytical scope to analytes which potentially have low probability to be present in the samples. The more commonly occurring analytes should continue to be measured using quantitative MRMs.

This report describes the screening validation of the QuEChERS method combined with a GC-QTOF from Agilent. The method was sought validated for 72 compounds in cereals at the three Screening Detection Limits, SDL, 0.01, 0.02 and 0.01 mg/kg.

The method validated here 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

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 ^{o}C for one hour. After another centrifugation at 5 ^{o}C 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 0.1 mg/kg with a mixture of pesticide standards and extracted by QuEChERS method. Part of the extracts was further diluted with blank cereals matrix (same type) to concentrations at 0.01 and 0.02. All extract were then analysed by analysed by GC-QTOF.

2.1 GC conditions

GC-system: 7890B, Agilent Technologies equipped with PAL auto sampler system and Gerstel PTV injector.

Injection volume: 5 µl

Injection programme: The injector was programmed from initially 30°C for 0.8 min to 290°C at a rate of 480°C per min and held for 2 min before further incensement to 310 °C at a rate of 720°C per min. Purge time was 3.05 minutes and aliquot of 5 µl extract was injected.

Column: Two HP5-5MS UI, 15 m, 0.25 mm ID, 0.25 µm df with backflush.

Oven programme: The oven was programmed from initially 60°C for 3 minutes increasing to 180°C at a rate of 30°C per min and then further, after 0.8 min increased to 290°C at a rate of 5°C min. This temperature was maintained for 16 min. Runtime: 38 min. Backflush at 300°C for 2.7 min.

2.2 TOF conditions

TOF instrument: 7200 GC/Q-TOF, Agilent Technologies

Ionisation mode: EI positive

Acquisition rate: 4GHz

Acquisition mode: centroid

Source temperature 230

Software: MassHunter B 07.00

2.3 PCDL library

Compounds analysed on GC, will most likely fragment in the ion source and often the molecular ion is therefore not detectable. Consequently, the analyses of the compounds must be performed on fragment ions. The PCDL software makes it possible to construct a library of spectra with exact masses. Currently, the EURL-CF has constructed a library with spectra for 81 compounds. Opposite Agilents Pesticide Spectrum Library, that contains measured spectra and consequently also ions not originating from the actual compound, this library contains only relevant ions. This library was used to build quantitative methods to process the data.

2.4 Processing of data

The MassHunter Qualitative Analysis, B.07.00 is not suitable for GC data processing. The software is clearly developed for LC analysis and still not satisfactory optimized for GC purposes. Consequently, the MassHunter Quantitave Analysis, B.07.01 was used instead. This software gives a good overview of the data. The masses used to identify the compounds were imported from the PCDL. Masses for up to five ions were imported and the software chooses the most intense ions (this could include the molecular ion). The method was then used to process a data set and the three best masses were kept and the other deleted. The ions were chosen based on mass accuracy, signal/noise and ion ratios. The masses of the ion included in the methods are listed in Table 1.

3 Validation plan

According to SANCO/12571/2013[2] the method should be validated on minimum 20 samples. Five samples of five different types of cereal samples were spiked. The samples were barley, rice, rye, oat and wheat, which were blank test materials from EUPT-C6, C5, C4, C2 and C2, respectively. The samples were spike with 4 standard mixtures called 1, 2, 7 and 8, containing 189 different compounds altogether. Not all the compounds included compound were GC amendable. The different cereal samples were spiked at 0.1 mg/kg and part of the extracts were further diluted with blank cereals matrix (same type) to concentrations at 0.01 and 0.02. All extract were then analysed by analysed by GC-QTOF. In total, 79 pesticides had been evaluated.

According to SANCO at least 95% of the samples should be detected (a false-negative rate of 5% is accepted). This means that only 1 out of 20 spiked samples are allowed to be non-detected.

4 Blank matrix

The barley, rice, rye and wheat used as test material were Blank Test Items from the proficiency test samples EUPT-C6, C5, C4, and C2, respectively.

5 Screening criteria

The screening criteria for the validation were set to the following values:

Parameter	Value
Retention time (RT)	± 0.1 min
Mass accuracy	10 ppm for at least 2 fragment ions
S/N	3
Ion ratios	30%

6 Validation results

The validation results are listed in the Table 1 together with the masses used for detection. The masses listed in the table are the positive exact masses of the fragments, e.i. the mass of the fragment minus the weight on one electron (0.0005 amu). Of the 72 compounds evaluated 65 compounds were validated, i.e. detected in minimum 95% of the spiked samples. Screening Detection Limit (SDL) of 0.01 mg/kg was obtained for 33 compounds, SDL of 0.02 mg/kg for 7 compounds and SDL 0.1 mg/kg for 23 compounds. The SDLs were defined as the lowest concentration at which a pesticide could be detected with a maximum of one non-detect out of the 25 samples.

Eighteen compounds were not validated. These include typically more LC amenable pesticides or more analytical challenging compounds, e.g. pesticides that are removed during clean-up. The compounds that were not validated are listed in Table 2.

No manual integration or extraction of masses was employed. All processing of data was done by Mass Hunter in the Quantitative software.

Table 1 Screening detection limits (SDL) and ion masses for validated pesticides. The masses of the positive ion were imported from Agilent Pesticide Spectrum Library or has been manually calculated. They are exact masses of the M^+ ions.

Pesticide	SDL mg/kg	Ion 1	Ion 2	Ion 3
2.3.5-Trimethacarb	0.01	136.0883	91.05423	121.0648
Alachlor	0.02	160.1121	188.107	146.0964
Allethrin	0.01	91.05423	105.0699	79.05423
Ametryn	0.01	227.1199	212.0964	185.073
Aminocarb	0.1	151.0992	136.0757	150.0913
Anthraquinone	0.1	180.057	151.0542	208.0519
Bifenox	0.1	309.9668	311.9641	173.0153
Bixafen	0.1	159.0364	413.0304	415.0276
Bromacil	0.01	204.9607	187.9342	206.9587
Butralin	0.01	266.1135	224.0666	220.1081
Buturon	0.02	152.9976	154.9946	125.0027
Captafol	0.1	79.05423	77.03858	80.06205
Carfentrazone-ethyl	0.01	310.0189	312.0591	340.0904
Chlorbufam	0.1	127.0183	152.9976	125.0027
Chlorthal-dimethyl	0.01	298.8831	220.8958	329.9015
Chlozolinate	0.1	186.9586	262.0033	170.9637
Coumaphos	0.01	362.0139	364.011	225.985
Cyanophos	0.01	243.0114	124.9821	109.0049
Cycluron	0.1	72.0444	89.0709	69.06988
Cyflufenamid	0.1	223.029	188.0118	240.0317
Demeton-S	0.02	88.03412	113.9535	114.9613
Desmetryn	0.01	213.1043	198.0808	171.0573
Dichlobenil	0.01	170.9637	172.9608	100.0182
Dichlofenthion	0.01	222.938	224.9354	279.0006
Difenoxuron	0.1	241.0733	226.0499	198.055
Dimethachlor	0.02	134.0964	197.0602	148.0757
Dioxacarb	0.02	121.0284	122.0362	166.0625
Edifenphos	0.02	109.0106	110.0185	172.9821
Esprocarb	0.01	91.0542	162.1277	222.0947
Ethalfluralin	0.01	276.0591	292.054	316.0904
Etofenprox	0.1	163.1117	135.0804	107.0491
Etoxazole	0.02	141.0146	300.1194	204.1383
Fenothiocarb	0.1	72.04439	94.04132	160.0791
Fenoxaprop-ethyl	0.01	288.0422	290.0392	361.0712
Fenpiclonil	0.01	235.9903	237.9873	201.0214
Flamprop-isopropyl	0.01	105.0335	276.0586	156.0011
Flonicamid	0.01	174.0161	146.0212	172.0243
Fluacrypyrim	0.01	145.0648	189.0546	204.0781
Flumioxazin	0.1	354.101	326.1061	259.0514
Furalaxyl	0.01	95.01276	152.0706	242.1176
Furathiocarb	0.01	163.0754	194.0396	107.0491
Furilazole	0.01	219.9927	221.9897	262.0032

Pesticide	SDL mg/kg	Ion 1	Ion 2	Ion 3
Furilazole	0.01	219.9927	221.9897	262.0032
Hexaflumuron	0.1	201.9457	302.9471	203.9428
Isodrin	0.01	192.9373	262.8564	264.8535
Isoxathion	0.01	105.0335	177.0243	130.0287
Lenacil	0.1	153.0659	234.1368	136.0393
Leptophos	0.01	171.0028	374.9006	376.8985
Mepronil	0.01	119.0491	269.141	210.0675
Methoprene	0.02	73.06479	111.0441	107.0855
Metolachlor	0.01	162.1277	238.0993	146.0964
Monuron	0.1	152.9976	154.9952	125.0027
Nitrofen	0.01	282.9798	284.9768	202.018
Novaluron	0.02	142.0054	308.9986	144.0025
Paraoxon-methyl	0.01	109.0049	230.0213	247.024
Pebulate	0.1	128.107	161.0869	72.04439
Permethrin,-cis	0.1	183.0804	163.0076	127.0309
Prometon	0.01	168.088	210.1349	183.1115
Propazine	0.01	214.0854	172.0384	229.1089
Pyrifenox I	0.01	262.0059	186.9586	170.9637
Quinoxyphen	0.01	237.0584	272.0273	306.9961
Sulprofos	0.1	156.0062	140.029	322.0279
Terbumeton	0.1	169.0958	210.1349	154.0723
Terbuthylazine	0.1	214.0854	173.0463	138.074
Tetramethrin	0.1	164.0706	107.0491	123.1168
Thiobencarb	0.1	100.0757	125.0153	257.0636

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

Pesticide	Ion 1	Ion 2	Ion 3
Chlorbromuron	232.9059	230.9081	234.9081
Dazomet	72.9981	71.99025	75.94359
Dimefuron	165.9928	167.9899	293.0562
Dinoseb	211.0349	193.0244	163.0264
Fluometuron	232.0818	159.029	187.024
Isoxaben	165.0546	150.0311	107.0128
Nicotine	84.08078	133.076	161.1073

7 Conclusions and future

Of the 72 compounds evaluated, 65 compounds were validated, 33 with SDL at 0.01 mg/kg, 9 with SDL at 0.02 mg/kg and 23 with SDL 0.1 mg/kg. In total, 7 compounds were not validated. All data was processed automatically with the quantitative software.

The validated pesticides fulfilled the following screening detection criteria, retention time could vary ± 0.1 min and the mass accuracy should be ≤10 ppm for at least 2 ions, molecular ion or fragment ions.

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.