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National Food Institute

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Screening Validation Report 9

Target screening method for pesticide residues in cereals using LC-Q-ToF-MS Maxis system from Bruker

Elena Hakme

Mette Erecius Poulsen

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1 Introduction

The target screening on LC-Q-ToF-MS is performed using full scan and Broadband Collision Induced Dissociation (bbCID) as data acquisition and screening against an accurate mass database of 942 compounds covering a wide range of pesticides for data processing.

This report describes the screening validation of the QuEChERS method combined with the LC-Q-ToF analysis. The method was validated for 62 analytes in cereals at three screening detection limits (SDLs) 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]. 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 [(2)].

2 Principle of analysis

2.1 Extraction method

The samples were extracted using the citrate-buffered QuEChERS (EN 15662) (CEN 2008) method without cleanup. Five g of sample were weighed. Procedural standard (dichlorvos-d6) was added to all samples before extraction. Then 10 mL of cold water was added, followed by 10 mL of acetonitrile. To aid the extraction, a ceramic homogenizer was used. The tubes were shaken for 1 min by hand. Next, 4.0 g of magnesium sulphate, 1.0 g of sodium chloride, 1.0 g of sodium citrate dehydrate, and 0.5 g of sodium citrate sesquihydrate were added. After 1 min of shaking by hand followed by centrifugation for 10 min at 4500 rpm, 8 mL of the supernatant was

transferred to a clean tube and stored at – 80°C for 1 h. The extracts were then thawed, and while they were still very cold, they were centrifuged at 4500 rpm for 5 min. Thereafter, 200 µL were diluted 1:1 with acetonitrile and filtered. Internal standard was added to the vials prior to injection. 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.

2.2 Instrumentation

LC conditions

The analysis were performed on an Ultimate 3000RS UHPLC focused from Thermo Fisher Scientific

Mobile phases: A (water + 0.1% formic acid + 5 mM ammoniac), B (methanol).

Column: InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm, narrow bore LC column

Eluent gradient:

Retention time (min)	Flow (mL/min)	% A	% B
0	0.2	90	10
0.5	0.2	90	10
3		55	45
14	0.4	5	95
16	0.4	5	95
16.1	0.4	90	10
19	0.2	90	10
20	0.2	90	10

Injection volume: 2µL

Total runtime: 20 min

QToF conditions

The analysis were performed on a MaxiII^{TM} ToF from Bruker

- Acquisition mode: Full scan mode with bbCID

The bbCID is a data acquisition process where both TOF MS full scan data and MS/MS fragments are continuously generated in two independent, rapidly alternating data channels, without either precursor ion or threshold selection. In the first channel, low collision energy is applied. Precursor ions are not isolated or fragmented and full scan TOF MS spectra is produced. In the second channel, bbCID is applied with high collision energy. All ions are fragmented in the collision cell resulting in bbCID MS/MS spectra.

- MS: Bruker impact QToF-MS
- Scan range: 30-1000 m/z
- Ionization: ESI, 2500V
- Positive mode

Softwares

Chromeleon Xpress is used for LC initialization.

Hystar version 3.2.44.0 from Bruker Daltonics is used for data acquisition.

OTOF control software 3.4 (Compass for otoSeries 1.7) is used for MS tuning and optimization. Calibration and tuning of the ToF instrument, ionization mode, ion transfer, tuning for high sensitivity/high resolution or wide mass range is performed using the OTOF software. The instrument uses internal lock-mass correction at m/z 622 to provide very high accuracy measurements. Drops of lock mass 622 is added to the sponge under a ceramic plate in the ion source. This calibration occurs during all the run. The instrument is also calibrated using soidum formate in positive mode and by direct infusion. This second calibration occurs during the 0.1-0.25 min at the beginning of the run.

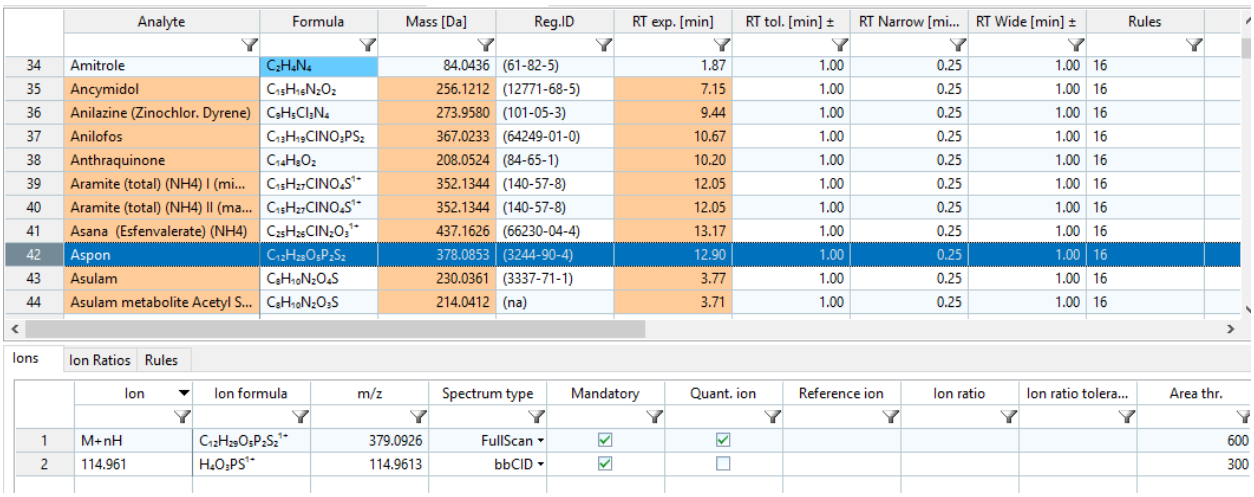
Bruker TASQ client 2.1 is used for data processing.

3 Validation plan

Samples of wheat, rye, oat, barley, and rice were spiked at three different concentration levels of 0.005, 0.01, and 0.02 mg/kg. Five replicates were prepared with each of the matrices and at each of the concentration levels. Thus, 25 cereal samples were spiked at each level, leaving us with 125 samples. Matrix matched-calibration was prepared using rye at the 5 concentration levels of 0.1, 0.033, 0.01, 0.0033, and 0.001 mg/kg.

4 Database

The database consists of 942 compounds. Each analyte has it is corresponding formula, mass (Da), cas number, retention time, with a retention time tolerance of 1 min. Strict narrow Rt of 0.25 min and wide Rt of 1 min are used for scoring. The principal ion used to generate determination is referred to as “M+nH” corresponding to a protonated ion. The ions generated from the FullScan are usually “M+nH” or “I” and the corresponding “M+nH+2” or “M+nH+1” or “I+1” isotopes. Additionally the database comprises ions generated from the bbCID. Each analyte has at least one ion from the bbCID. In total, each analyte has a minimum of 2 fragments ions.



	Analyte	Formula	Mass [Da]	Reg.ID	RT exp. [min]	RT tol. [min] ±	RT Narrow [mi...	RT Wide [min] ±	Rules
34	Amitrole	$C_2H_4N_4$	84.0436	(61-82-5)	1.87	1.00	0.25	1.00	16
35	Ancymidol	$C_{15}H_{16}N_2O_2$	256.1212	(12771-68-5)	7.15	1.00	0.25	1.00	16
36	Anilazine (Zinochlor. Dyrene)	$C_9H_5Cl_3N_4$	273.9580	(101-05-3)	9.44	1.00	0.25	1.00	16
37	Anilofos	$C_{13}H_{19}ClNO_3PS_2$	367.0233	(64249-01-0)	10.67	1.00	0.25	1.00	16
38	Anthraquinone	$C_{14}H_8O_2$	208.0524	(84-65-1)	10.20	1.00	0.25	1.00	16
39	Aramite (total) (NH4) I (mi...	$C_{15}H_{27}ClNO_4S^{1+}$	352.1344	(140-57-8)	12.05	1.00	0.25	1.00	16
40	Aramite (total) (NH4) II (ma...	$C_{15}H_{27}ClNO_4S^{1+}$	352.1344	(140-57-8)	12.05	1.00	0.25	1.00	16
41	Asana (Esfenvalerate) (NH4)	$C_{25}H_{28}ClN_2O_3^{1+}$	437.1626	(66230-04-4)	13.17	1.00	0.25	1.00	16
42	Aspon	$C_{12}H_{28}O_5P_2S_2$	378.0853	(3244-90-4)	12.90	1.00	0.25	1.00	16
43	Asulam	$C_8H_{10}N_2O_4S$	230.0361	(3337-71-1)	3.77	1.00	0.25	1.00	16
44	Asulam metabolite Acetyl S...	$C_8H_{10}N_2O_3S$	214.0412	(na)	3.71	1.00	0.25	1.00	16

Ions | Ion Ratios | Rules

	Ion	Ion formula	m/z	Spectrum type	Mandatory	Quant. ion	Reference ion	Ion ratio	Ion ratio tolera...	Area thr.
1	M+nH	$C_{12}H_{29}O_5P_2S_2^{1+}$	379.0926	FullScan	☑	☑				600
2	114.961	$H_4O_3PS^{1+}$	114.9613	bbCID	☑	☐				300

Figure 1: LC-Q-ToF Database screenshot figure from the TASQ software.

5 Data Processing

Data were processed using the **Target Analysis for Screening and Quantitation** (TASQ) software. The target screening was performed against the database with a signal to noise filter of 3, mass error set to 2 ppm, and m/z values set to 4 (number of digits for results representing mz). Principal ion and at least one mandatory ion is selected for determination.

For each analyte (including its qualifiers) an individual score is displayed and summarized as MRSQ score. The capital letters stand for mass accuracy (M), retention time (R), isotope pattern fit (mSigma value; S), and qualifier ions (presence and ion ratio quality; Q). MRSQ are used to assign a rating and color to each of the mentioned parameters.
The retention time accuracy is measured by the difference between the expected and measured retention time. The mass accuracy is measured by the difference between the calculated and measured m/z value (in mDa or ppm). The isotopic peak pattern fit (mSigma value) is a quality factor obtained from the difference between the theoretical and measured isotopic patterns. Additionally, the presence or not of qualifiers with ion ratio values are also displayed. Therefore, screening result review is supported by simplified color coding of matched parameters.

Therefore, all the following parameters were adjusted in the database by injecting and processing a mix of standard before any spiking sample is analysed and processed. Linearity is checked in the range of 0.1-0.001 mg/kg.

6 Conclusions

The 62 pesticides included in this study were successfully validated for screening. The majority (47 pesticides) showed a SDL of 0.005 mg/kg. An SDL of 0.01 mg/kg was established for 12 compounds and only 3 compounds showed a SDL of 0.05 mg/kg.

7 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), retention time, and the exact masses of principal ions, isotopic ions, and at least 1 fragment ion (bbCID) for each compound.

Compounds	SDL (mg/kg)	Rt (min)	Full scan prinicpal ion (M+nH or I)	Isotopic ion (M+nH+1 or M+nH+2 or I+2)	bbCID ion 1	bbCID ion 2	bbCID ion 3
2-6-Dichlorobenzamide	0.01	4.28	189.9821	191.9792	172.9555		
Allidochlor	0.01	5.82	174.0680	176.0652	98.0964		
Ancymidol	0.005	6.98	257.1288		135.0441	81.0447	
Anilofos	0.01	10.52	368.0305	370.0277	124.9821		
Aramite	0.005	11.89/12.05	352.1344	354.1318	191.1430		
Aspon	0.005	12.74	379.0926		114.9613		
Atrazine-desethyl	0.005	5.3	188.0697	190.9669	146.023	104.001	79.0058
Azaconazole	0.005	8	300.0301	302.0273	158.976		
Aziprotryne	0.005	9	226.0869		89.0168	156.0338	
Beflubutamid	0.01	10.29	356.1268		91.0542		
Benodanil	0.005	7.46	323.9877		230.9301		
Benoxacor	0.005	8.18	260.0240	262.0212	149.0835	120.0444	
Benzoximate	0.005	10.92	199.0156	201.013	199.0156		
Benzoylprop-ethyl	0.005	10.68	366.0658	368.0632	105.0335		
Butachlor	0.005	12.18	238.0993	240.0967	162.128	147.1043	
Butafenacil	0.005	9.59	492.1144	494.1123	331.0092		
Butamifos	0.005	10.85	333.1032		95.9667		
Butylate	0.01	11.61	218.1573	219.1603			
Chlordimeform	0.005	4.34	197.0821		117.0573		
Chlorimuron-ethyl	0.005	9.19	415.0474	417.0449	186.0065	184.9903	278.0094

Chloroxuron	0.005	9.46	291.0895	293.0895	72.0444		
Clothianidin	0.02	4.74	250.0160	252.0130	131.9669	113.0168	
Crimidine	0.01	5.22	172.0636	174.0607	136.0869		
Daimuron	0.01	9.24	269.1648		151.0866	108.0808	
Dichlormid	0.005	6.55	208.029	210.0262	41.0386	98.0964	81.0699
Diclobutrazol	0.005	10.32	328.0978	330.0951	70.0400		
Dimethylvinphos_Z	0.005	9.43	330.9455	332.9427	127.0155		
Diphenamid	0.005	8.07	240.1383		134.0964		
Dithiopyr	0.005	11.41	402.0615		354.0582		
Etaconazole	0.005	9.79	328.0614	330.0587	158.9763		
Ethiprole	0.005	8.85	396.9899	398.9870	350.9480		
Famphur	0.005	7.53	326.028		93.0100		
Fenfuram	0.005	6.93	202.0863		109.0284		
Fenobucarb	0.005	8.48	208.1332		95.0491		
Flucycloxuron	0.005	12.64	484.1234	486.1216	132.0444		
Fluoroglycofen-ethyl	0.005	11.49	465.0671	467.0650	343.9932		
Fluridone	0.005	8.42	330.1100		310.1038		
Halofenozide	0.005	8.81	105.0335		105.0335		
Hexazinone	0.01	6.64	253.1659		171.0877		
Imazamethabenz-methyl	0.005	6.26	289.1547		86.0964	144.0444	
Imibenconazole	0.005	12.15	410.9999	412.9971	125.0153		
Inabenfide	0.02	8.76	339.0895	341.0872	321.0789	80.0495	
Iprobenfos	0.01	10.3	289.1022		91.05420		
Isazophos	0.005	9.33	314.0490	316.0461	119.9959		
Isocarbamid	0.005	5.38	186.1237		87.0553		
Isocarbophos	0.01	7.83	273.0345		121.0287		
Isoxadifen-ethyl	0.005	10.21	296.1281		204.0808	232.0757	
Lethane	0.01	7.49	204.1053		104.0165		
Mefenpyr-diethyl	0.005	10.73	373.0716	375.069	327.0298		
Methabenzthiazuron	0.02	7.6	222.0696		165.0481		
Norflurazon	0.005	7.97	304.0459	306.0432	284.0397		
Pretilachlor	0.005	11.55	312.1725	314.1701	252.115	176.1434	
Propaphos	0.005	10.51	305.0971	306.1003			
Rabenzazole	0.005	7.55	213.1135		172.0869		
Siduron	0.005	8.71/8.91	233.1648		137.0709	94.0651	
Tebupirimfos	0.005	11.9	319.1240		277.0770	153.1022	231.0352
Tebutam	0.005	9.9	234.1852	235.1885			
Thenylchlor	0.005	9.75	324.0820	326.0793	127.0212		
Thiazopyr	0.005	10.35	397.1004		335.0472	377.0941	
Tiocarbazil	0.005	12.57	280.1730		91.0542		
Tolfenpyrad	0.01	12.13	384.1473	386.1452	197.0961		
Tribufos	0.005	13.37	315.1034		168.9905	112.9279	