DTU Food
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Screening method validation of pesticide residues in cereals using GC-QTOF

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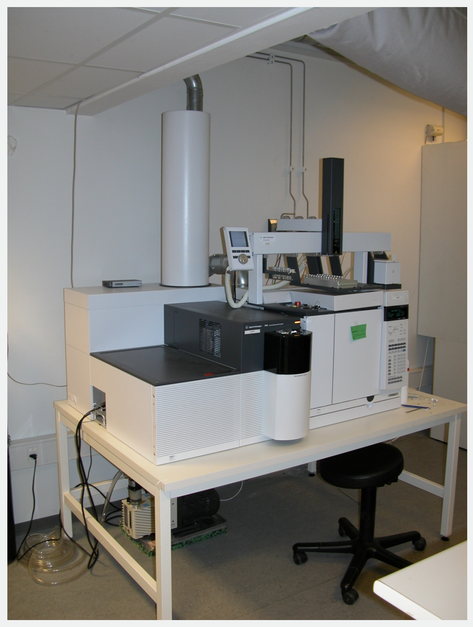

Foto: GC-QTOF DTU National Food Institute

Instrumentation
QTOF: 7200 GC/Q-TOF, Agilent Technologies

GC: Agilent 7890A GC with back flush Gerstel PTV injector and CTC autosampler.

Columns: Two HP-5MS UI, 15 m, 0.250 mm diameter, 0.25 mm film thickness.

Software: Agilent Mass Hunter Version B.06.00

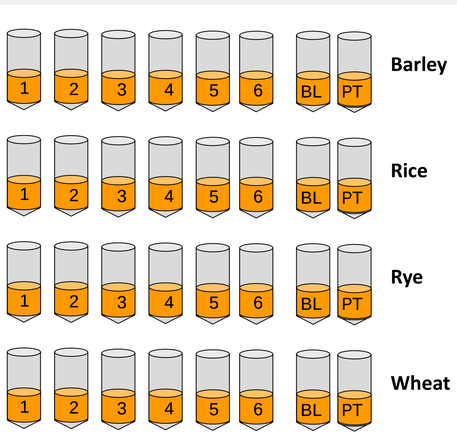

Figure 2: Extraction of spiked samples, blanks and PT test materials

Table 1: Typical examples on validation data. Pink colored numbers are validated spike level.

Spike level, mg/kg		0.01	0.01	0.05	0.05	
	Molecular or fragment	No. of positive samples out of 24				
Mass accuracy difference	ion*	≤5ppm	≤10ppm	≤5ppm	≤10ppm	SDL, mg/kg
Bifenthrin	1	19	24	24	24	0.01
Bifenthrin	2	4	24	3	24	
Boscalid	1	5	24	6	24	0.05
Boscalid	2	1	2	13	24	
Boscalid	3	4	11	0	19	
Carboxin	1	15	22	24	24	0.05
Carboxin	2	6	20	3	24	
Chlorpyrifos	1	20	24	24	24	0.01
Chlorpyrifos	2	20	24	24	24	
Chlorpyrifos	3	22	24	24	24	
Chlorpyrifos-methyl	1	24	24	24	24	0.01
Chlorpyrifos-methyl	2	20	22	24	24	
Cypermethrin	1	8	20	17	24	0.05
Cypermethrin	2	10	22	6	24	
Cyprodinil	1	4	22	0	24	0.05
Cyprodinil	2	21	24	24	24	
Diazinon	1	21	24	24	24	0.01
Diazinon	2	16	24	24	24	
Diclorvos	1	14	24	23	24	0.05
Diclorvos	2	4	12	0	21	
Difenoconazole	1	10	21	24	24	0.05
Difenoconazole	2	3	14	23	24	
Fenitrothion	1	19	24	24	24	0.01
Fenitrothion	2	22	24	24	24	
Fenitrothion	3	20	24	24	24	
Fipronil	1	21	24	24	24	0.01
Fipronil	2	22	24	24	24	

Introduction: GC-QTOF is a new detection technique in the field of screening for pesticides residue in food samples. The technique will enable screening of many pesticides compared to the MS/MS analyses commonly used. With the purpose to develop generic screening methods, the EURL-CF has purchased an Agilent QTOF 7200. Information on instrumentation can be seen in the box to the left.

Library : GC-QTOF presents other challenges than LC-QTOF, especially because the molecular ions typically do not survive. The compounds are fragmented in the ion source and currently no libraries with exact masses of the fragments are available. The EURL-CF will in cooperation with the EURL-FV create a library with exact masses of GC amendable pesticides. MS-interpreter in the NIST MS Library is one of the tools to identify the fragments and exact masses, see Figure 1

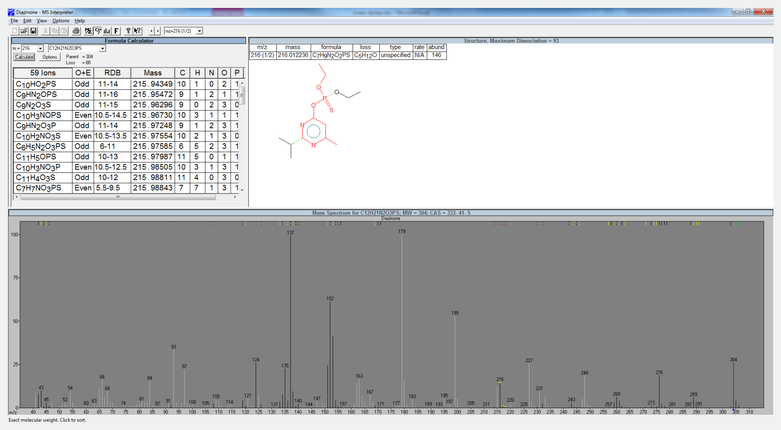

Figure 1 Screen dump of NIST library MS-interpretor for diazinon

Method: Barley, rice, rye and wheat cereal samples with no pesticide residues were spiked at 0.01 and 0.05 mg/kg with a mixture of more than 300 pesticides. Six replicates of each cereal type was spiked (Figure 2). The samples were extracted by QuEChERS method and analysed by GC-QTOF. Together with the 24 spiked samples, 4 blank cereal samples (same cereals types) and 4 EUPT tests materials were extracted and cleaned up. Only 38 of the compounds have currently been evaluated.

Validation criteria: According to SANCO at least 95% of the recovery samples should be detected, meaning that only 1 out of 20 spiked samples are allowed to be non-detected. The criteria to detect a compound in this validations was a slightly different from those listed in SANCO/12571/2013.
See Figure 3

Validation - spike: A screen dump of pirimiphos-methyl result showing the software and chromatogram at 0.01 mg/kg is shown in Figure 4. The validation results showed that of 36 of the 38 evaluated compounds was validated, 19 with Screening Detection Limit, SDL, at 0.05 mg/kg and 17 with SDL 0.01 mg/kg. The SDL was defined as the lowest concentration in which a pesticide could be detected with only one non-detect or less out of the 24 samples. All processing of data was done automatically by Mass Hunter in the Quantitative software. No manual assessment was done. See Table 1.

EUPT-C test materials: Results from the EUPT test materials showed very good agreement with the validation. The test materials contained 46 residues of 27 different pesticides in the range of 0.012-2.180 mg/kg. All pesticides were detected apart from one residue of lambda-cyhalothrin. However, this residue was below the SDL of 0.05 mg/kg. No false positives were seen. See Table 2

- ❖DTU:
  - Retention time (RT): ± 0.1 min
  - Signal to noise ratio (S/N): 6
  - Mass accuracy:
    - 5 ppm for at least 1 fragment ion or
    - 10 ppm for at least 2 fragment ions
- ❖SANCO/12571/2013:
  - Retention time (RT): ±0.2 min
  - Signal to noise ratio (S/N): 3
  - ≥ 2 diagnostic ions, preferably including the (quasi) molecular ion;
  - mass accuracy < 5 ppm; at least one fragment ion

Figure 3 Criteria for detection used in this validation (DTU) compared to SANCO /12571/2013 .

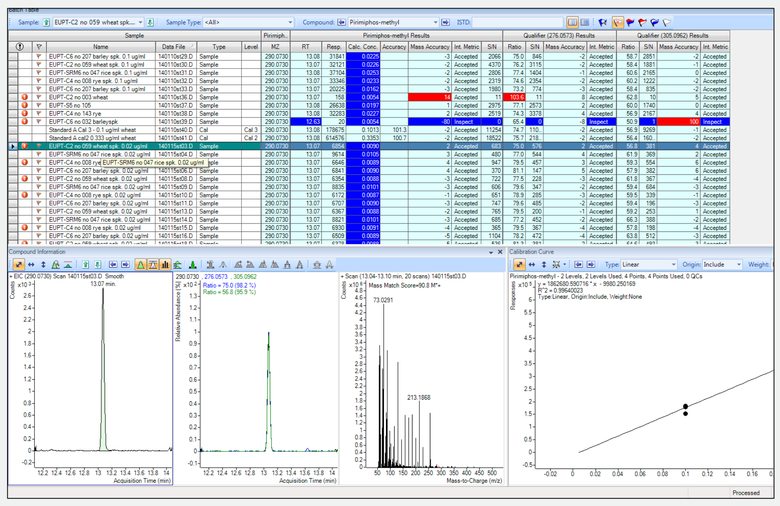

Figure 4: Screen dump of quantitative software for pirimiphos-methyl at spike level 0.01 mg/kg

Table 2: Screening result s of EUPT-C2, -C4, -C5 and -C6.

Azinphos-methyl	EUPT-C2 wheat	Assigned values, mg/kg	EUPT-C5 Rice	Assigned values, mg/kg	EUPT-C4 rye	Assigned values, mg/kg	EUPT-C6 barley	Assigned values, mg/kg
Azoxystrobin	D	0.239	D	0.164	D	0.316	D	0.196
Bifenthrin	D	0.087						
Boscalid							D	0.910
Carboxin							D	0.144
Chlorpyrifos			D	0.1985			D	0.173
Chlorpyrifos-methyl	D	0.13			D	0.125		
Cypermethrin	D	0.098					D	0.284
Cyprodinil							D	0.150
Diazinon								
Diclorvos								
Difenoconazole	D	0.169	D	0.1				
Epoxiconazole	D	0.176	D	0.0966			D	0.594
Fenbuconazole								
Fenitrothion					D	0.188		
Fipronil			D	0.1525				
Flutriafol					D	2.18		
Iprodione	D	0.289						
Krexoxim-methyl			D	0.168	D	0.396		
Lambda-cyhalothrin			ND	0.025	D	0.065		
Malathion	D	0.168	D	0.012	D	0.108		
Metconazole								
Methacrifos								
Penconazole								
Pendimethanil							D	0.108
Pirimicarb	D	0.038					D	0.252
Pirimiphos-methyl			D	0.0735	D	0.078		
Prochloraz	D	0.239						
Procymidone								
Propiconazole			D	0.442			D	0.206
Pyraclostrobin							D	0.473
Spiroxamin	D	0.075			D	1.1		
Tebuconazole			D	0.813			D	0.431
Triademenol					D	1.62		
Trifloxystrobin	D	0.439	D	0.216				
Trifluralin								
Triticonazole								
Vinclozolin								

[1] **EURL-CF: EU Reference Laboratory for pesticide Residues in Cereals and Feeding stuff . DTU National Food Institute, Moerkhoej Bygade 19, DK-2860 Soeborg, Denmark**
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