CRL for Cereals and Feeding stuff
National Food Institute
Technical University of Denmark

# Validation Report 6

**Determination of pesticide residues in cereals by GC-MS/MS and LC-MS/MS**

**(QuEChERS method)**

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**CONTENT:**



## 1. Introduction

This report describes the validation of the QuEChERS method combined with GC-MS/MS and LC-MS/MS. The method was sought validated for 55 pesticides, isomers and degradation products in wheat, rye and oat. The method has previously been validated for about 98 pesticides.

The QuEChERS method has an extraction and clean-up step, which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit and vegetables[1].

## 2. Principle of analysis

**Sample preparation:**

Cold water/ice water and acetonitril are added to the milled sample.

**Extraction:**

The sample is shaken and a salt and buffer mixture is added and the sample is shaken again.

Clean-up:

After centrifugation the supernatant is transferred to a tube with PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract is obtained.

For the LC-MS/MS analysis the extraction is followed by adding internal standard and the extract is filtered into HPLC vials.

**Quantification and qualification**:

GC-MS/MS:

The final extract is analysed GC/MS/MS (electron energy 70 eV, source temp. 180°C, transfer line GC interface 250°C). The injection volume is 4 µl.

LC-MS/MS:

The pesticide residues are separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI).

**Selectivity and specificity:**

GC-MS/MS:

GC-MS/MS is a highly selective method, and thereby highly specific. Two MRM transitions were used (two parent and two daughter ion) one for quantification and another transition for qualification. Parent and daughter ions are presented in appendix 1.

LC-MS/MS:

The validation includes pesticides determined with both positive and negative ESI. $^{13}C_6$-carbaryl was used as internal standard for quantification. All pesticides were detected in the multiple reaction monitoring mode (MRM). For each pesticide precursor ion and 2 product ions (where possible) were determined. One product ion for quantification and one for qualification. The MRM transitions for the pesticides and degradation products sought validated are given in appendix 1.

## 3. Validation design

The method was south validated for 55 pesticides, isomers or degradation products (appendix 1) in wheat, rye and oat. The validation was performed on 5-6 replicates at each of the three concentration levels. The concentration levels were 0.01, 0.02 and 0.1 mg/kg. A blank sample was included. The tests were done on different days by two different technicians.

## 4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels, i.e. 0.003, 0.01, 0.033, 0.1 and 0.333 µg/ml. The calibration curves were best fitted to a linear curve. The quantification was performed from the mean of two calibration curves surrounding the samples. The majority of the correlation coefficients (R) were higher or equal to 0.98. Examples of chromatograms obtained when analysing the extracts by GC-MS/MS are presented in figure 1. Examples of calibration curves are presented in Figure 2.

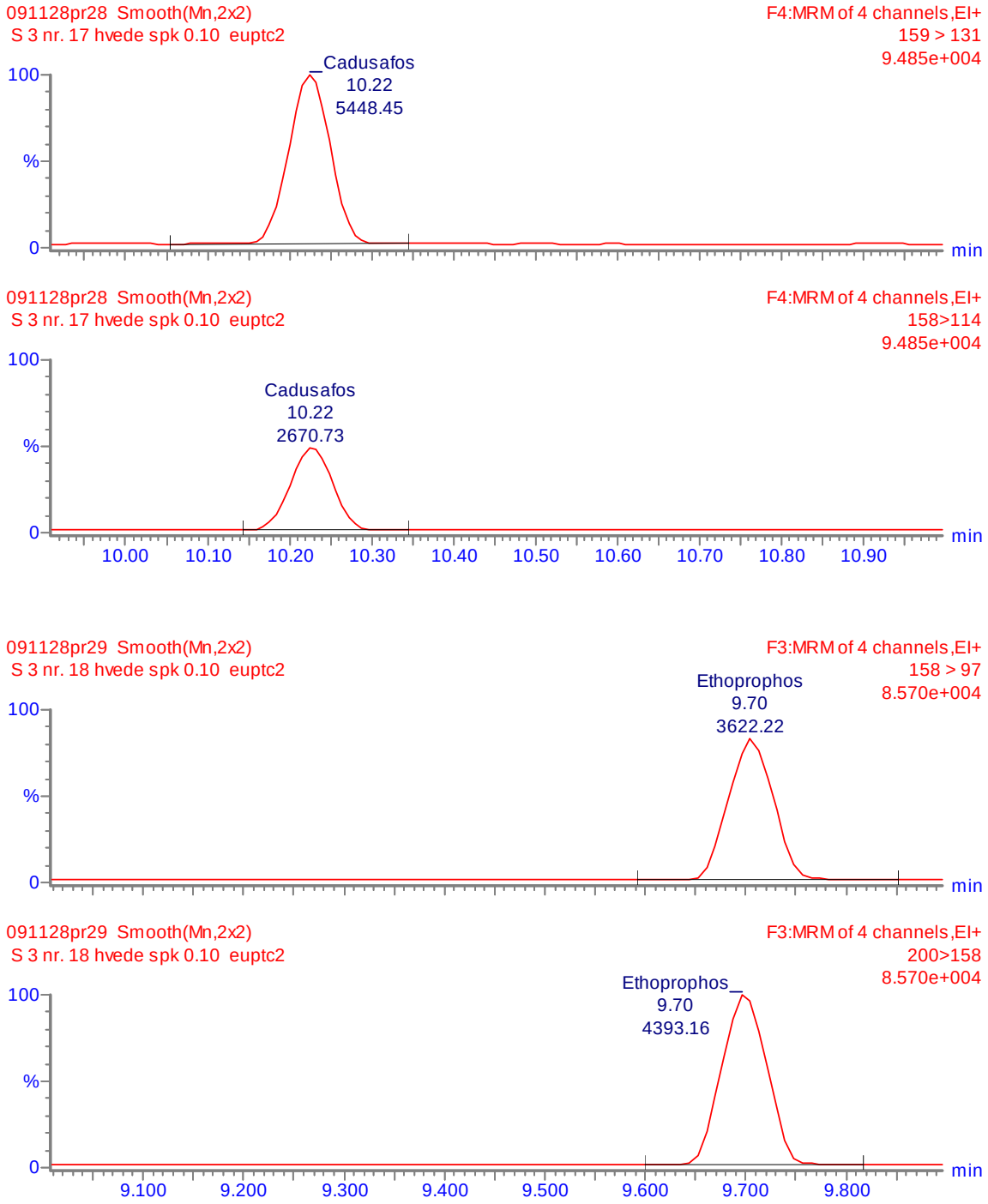
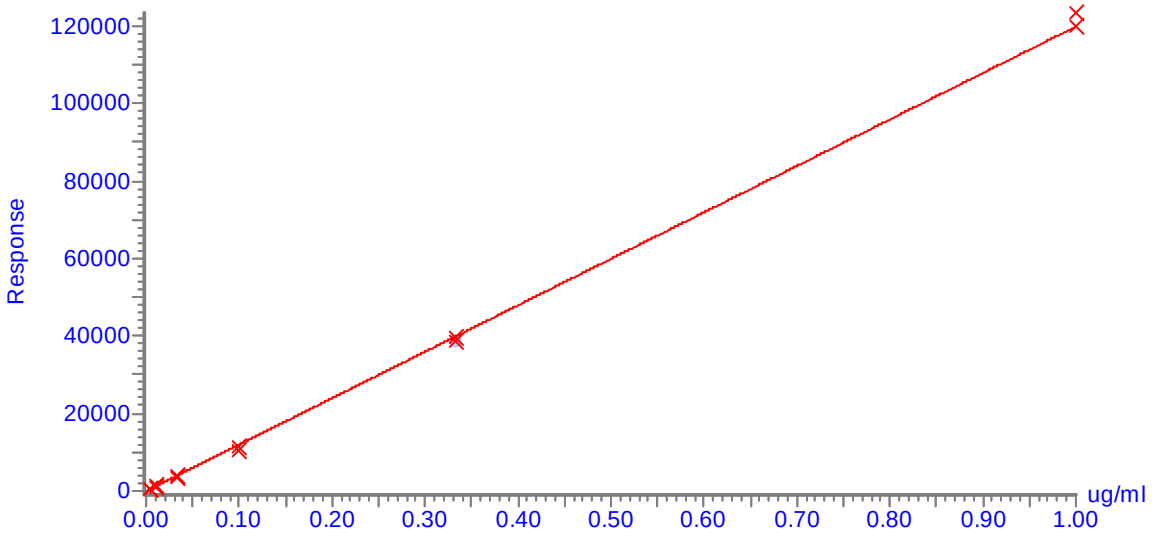



**Figure 1:** Examples of chromatograms for cadusafos and ethoprophos obtained when analysing extract of wheat spiked with 0.1 mg/kg.

Compound name: Cadusafos
Correlation coefficient: r = 0.999307, r^2 = 0.998614
Calibration curve: 119913 * x + -69.5052
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None




Compound name: Ethoprophos
Correlation coefficient: r = 0.999023, r^2 = 0.998047
Calibration curve: 78907 * x + -74.5637
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

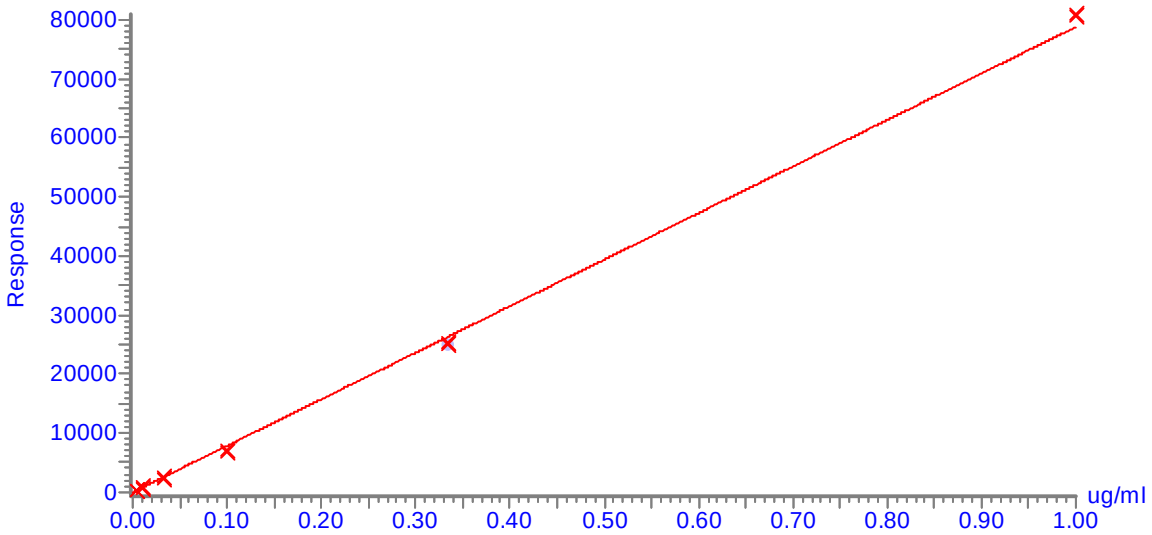



**Figure 2.** Examples of calibration curves for cadusafos and ethoprophos (concentrations from 0.003-0.333 µg/ml)

## 5. Validation parameters

**Precision – Repeatability**
Repeatability was calculated for all pesticides and degradation products on all three spiking levels.

Repeatability is given as the relative standard deviation on the result from two or more analysis at the same sample, done by the same technician, on the same instrument and within a short period of time. Repeatability in this validation was calculated from the 5-6 replicate determinations.

Repeatability were calculated as given in ISO 5725-2[2].

Appendix 2 and 3 shows the relative repeatability for the validated pesticides, isomers and degradation products.

**Accuracy – Recovery**
The accuracy was determined by recovery, samples were spiked at three concentration levels. In appendix 2 and 3 recovery, repeatability and limit of quantification (LOQ) are given for the validated pesticides, isomers and degradation products for all three concentration levels (0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg). Recoveries may be seen in appendix 2 and 3.

**Robustness**
The QuEChERS method has earlier by Anastassiades et al. 2003[1] in connection with the development of the method been shown to be robust.

**Limit of quantification, LOQ**
Quantification limits (LOQ) are calculated from the results at the lowest accepted spike level, as 6 times the standard deviation (absolute recovery). The quantification limits are given in appendix 2 and 3.

## 6. Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:

1. The relative standard deviation of the repeatability must be less than or equal to the standard deviation proposed by Horwitz[3].
2. The average relative recovery must be between 70 and 120%[4].

If the above mentioned criteria have been meet, the detection limits have been calculated.

# 7. Results and discussion

## LC-MS-MS compounds

The multi-residue method has been tested for 50 pesticides, isomers and degradation products in wheat, rye, and oat using LC-MS/MS. The relative repeatability ($RSD_r$) varied between 2-22 %, however most of the values were around 6-14%. For the majority of the pesticides the recovery was in the range of 75-100% at all three concentration levels.

For wheat the criteria for acceptance were met for 32 out of 50 pesticides, isomers and degradation products (listed in Table 1). For rye and oat the criteria for acceptance were met for 34 out of 50 pesticides, isomers and degradation products (listed in Table 2-3). The lowest calibration level (LCL) was 0.0033 µg/ml and most of the LOQs are above 0.010 mg/kg.

The pesticides, isomers and degradation product which has been validated presented in table 1-3 are divided in to two groups, one for the compounds for which the acceptance criteria could be meet (Accepted) and those which could not meet the acceptance criteria (Not accepted).

The results for the different pesticides which were accepted for LC-MS/MS are listed in Appendix 2.

**Table 1**: Compounds validated and not accepted as validated for wheat.

| **Wheat** | | |
|---|---|---|
| **Accepted (32 compounds)** | | |
| Avermectin B | Fenthionsulfon | Pencycuron |
| Boscalid | Fenthionsulfoxid | Phosmet |
| Bromuconazole sum | Fipronil | Phoxim |
| Cadusafos | Fipronil sulfone | Propagite |
| Clofentezin | Fipronil-sulfide | Spinosad A |
| Clothianidin | Formetanate | Tebufenozide |
| Ethoprophos | Fosthiazate | Teflubenzuron |
| Etofenprox | Lufenuron | Thiacloprid |
| Fenamiphos | Mepanipyrim | Thiamethoxam |
| Fenamiphos sulphone | Methoxyfenozide | Triazophos |
| Fenoxycarb | Paclobutrazole | |
| **Not accepted (18 compounds)** | | |
| Amitraz | Fenthion | N-2,4-dimethylphenylformamide |
| Diafenthiurone | Flufenoxuron | 2.4 dimethylphenyl-N-methylformamidine |
| Dinocap | Imidacloprid | Pyrethrins |
| Diphenylamine | Indoxacarb | Tolcloflos-methyl |
| Fenamidon | Prothioconazole | Zoxamide |
| Fenamiphos sulfoxide | Prothioconazole desthio | Spinosad D |

**Table 2**: Compounds validated and not accepted as validated for oat.

| **Oat** | | |
|---|---|---|
| **Accepted (34 compounds)** | | |
| Avermectin B | Fipronil | Propagite |
| Boscalid | Fipronil sulfone | Spinosad A |
| Bromuconazole sum | Fipronil-sulfide | Spinosad D |
| Cadusafos | Formetanate | Tebufenozide |
| Clofentezin | Fosthiazate | Teflubenzuron |
| Clothianidin | Lufenuron | Thiacloprid |
| Diphenylamine | Mepanipyrim | Thiamethoxam |
| Ethoprophos | Methoxyfenozide | Tolcloflos-methyl |
| Etofenprox | Paclobutrazole | Triazophos |
| Fenamiphos | Pencycuron | Zoxamide |
| Fenamiphos sulphone | Phosmet | |
| Fenoxycarb | Phoxim | |
| **Not accepted (16 compounds)** | | |
| Amitraz | Fenthionsulfon | N-2,4-dimethylphenylformamide |
| Diafenthiurone | Fenthionsulfoxid | Prothioconazole desthio |
| Dinocap | Flufenoxuron | Pyrethrins |
| Fenamidon | Imidacloprid | 2.4 dimethylphenyl-N-methylformamidine |
| Fenamiphos sulfoxide | Indoxacarb | |
| Fenthion | Prothioconazole | |

**Table 3**: Compounds validated and not accepted as validated for rye.

| **Rye** | | |
|---|---|---|
| **Accepted (34 compounds)** | | |
| Boscalid | Fipronil | Propagite |
| Bromuconazole sum | Fipronil sulfone | Spinosad A |
| Cadusafos | Fipronil-sulfide | Spinosad D |
| Clofentezin | Formetanate | Tebufenozide |
| Clothianidin | Fosthiazate | Teflubenzuron |
| Diphenylamine | Lufenuron | Thiacloprid |
| Ethoprophos | Mepanipyrim | Thiamethoxam |
| Etofenprox | Methoxyfenozide | Tolcloflos-methyl |
| Fenamiphos | Paclobutrazole | Triazophos |
| Fenamiphos sulphone | Pencycuron | Zoxamide |
| Fenoxycarb | Phosmet | |
| Fenthionsulfoxid | Phoxim | |
| **Not accepted (16 compounds)** | | |
| Amitraz | Fenthion | N-2,4-dimethylphenylformamide |
| Avermectin B | Fenthionsulfon | 2.4 dimethylphenyl-N-methylformamidine |
| Diafenthiurone | Flufenoxuron | Prothioconazole desthio |
| Dinocap | Imidacloprid | Pyrethrins |
| Fenamidon | Indoxacarb | |
| Fenamiphos sulfoxide | Prothioconazole | |

**GC-MS-MS compounds**

The multi-residue method has been tested for 32 pesticides, isomers and degradation products in wheat, rye, and oat using GC-MS/MS. The relative repeatability ($RSD_r$) varied between 2-20 %, however most of the values were below 15 %. For the majority of the pesticides the recovery was in the range of 75-100% at all three concentration levels.

For wheat and oat the criteria for acceptance were met for 27 out of 32 pesticides, isomers and degradation products (listed in Table 4-5). For rye the criteria for acceptance were met for 28 out of 32 pesticides, isomers and degradation products (listed in Table 6).

The pesticides, isomers and degradation product which has been validated presented in table 4-6. The results for the different pesticides which were accepted are listed in Appendix 3.

**Table 4**: Compounds validated and not accepted as validated for wheat.

| **Wheat** | | |
|---|---|---|
| **Accepted (27 compounds)** | | |
| Boscalid | Fenamiphos-sulfon | Indoxacarb |
| Bromuconazole sum | Fenthion | Mepanipyrim |
| Cadusafos | Fenthion-sulfon | Paclobutrazole |
| Chlorfenapyr | Fenthion-sulfoxid | Pencycuron |
| Clofentezin | Fipronil | Phosmet |
| Diphenylamine | Fipronil-sulfide | Tefluthrin |
| Ethoprophos | Fipronil-sulfone | Thiamethoxam |
| Etofenprox | Flufenoxuron | Tolclofos-methyl |
| Fenamiphos | Fosthiazate sum | Triazophos |
| **Not accepted (5 compounds)** | | |
| Lufenuron | Propargite sum | Amitraz |
| Formetanate | Fenamiphos-sulfoxide | |

**Table 5**: Compounds validated and not accepted as validated for oat.

| **Oat** | | |
|---|---|---|
| **Accepted (27 compounds)** | | |
| Boscalid | Fenamiphos-sulfoxide | Lufenuron |
| Bromuconazole sum | Fenthion | Mepanipyrim |
| Cadusafos | Fenthion-sulfon | Paclobutrazole |
| Chlorfenapyr | Fenthion-sulfoxid | Pencycuron |
| Clofentezin | Fipronil | Phosmet |
| Ethoprophos | Fipronil-sulfide | Tefluthrin |
| Etofenprox | Fipronil-sulfone | Thiamethoxam |
| Fenamiphos | Flufenoxuron | Tolclofos-methyl |
| Fenamiphos-sulfon | Indoxacarb | Triazophos |
| **Not accepted (5 compounds)** | | |
| Amitraz | Formetanate | Propargite sum |
| Diphenylamine | Fosthiazate sum | |

**Table 6**: Compounds validated and not accepted as validated for rye.

| **Rye** | | |
|---|---|---|
| **Accepted (28 compounds)** | | |
| Boscalid | Fenamiphos-sulfoxide | Mepanipyrim |
| Bromuconazole sum | Fenthion | Paclobutrazole |
| Cadusafos | Fenthion-sulfon | Pencycuron |
| Chlorfenapyr | Fenthion-sulfoxid | Phosmet |
| Clofentezin | Fipronil | Tefluthrin |
| Diphenylamine | Fipronil-sulfide | Thiamethoxam |
| Ethoprophos | Fipronil-sulfone | Tolclofos-methyl |
| Etofenprox | Flufenoxuron | Triazophos |
| Fenamiphos | Fosthiazate sum | |
| Fenamiphos-sulfon | Indoxacarb | |
| **Not accepted (4 compounds)** | | |
| Amitraz | Lufenuron | Propargite sum |
| Formetanate | | |

## 8. Conclusions

In conclusion 44 of 55 pesticides, isomers and degradation products were validated for the QuEChERS method using GC-MS/MS and LC-MS/MS for the analysis. 25 compounds were validated on both GC-MS/MS and LC-MS/MS. 13 compounds were only validated on LC-MS/MS and 6 compounds were only validated on GC-MS/MS.

## 9. References

**1** http://www.quechers.com/ or Anastassiades et al., J. AOAC Int., vol. 86, no. 2, p. 412, 2003

**2** ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part 2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

**3** W. Horwitz, Anal. Chem., 1982; 54, 67A.

**4** Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/10684/2010, 01/01/2010, European Commission, Brussels, 2010.

# Appendix 1. MRM transitions for the pesticides sought validated.

| GC-MS/MS | | Parent 1 | Fragment 1 | Col. energi 1 | Intensity | Parent 2 | Fragment 2 | Col. energi 2 | Intensity |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Clofentezin | 137 | 102 | 10 | 53600 | 102 | 75 | 10 | 58800 |
| 2 | Lufenuron | 352.9 | 203 | 15 | 677000 | 173.9 | 110 | 10 | 329000 |
| 3 | Diphenylamine | 169 | 168 | 10 | 7750000 | 168 | 167 | 10 | 6450000 |
| 4 | Ethoprophos | 158 | 97 | 15 | 12300000 | 200 | 158 | 5 | 13400000 |
| 5 | Pencycuron | 180 | 125 | 10 | 7880000 | 125 | 89 | 10 | 1370000 |
| 6 | Cadusafos | 159 | 131 | 5 | 18800000 | 158 | 114 | 5 | 6680000 |
| 7 | Flufenoxuron | 331 | 268 | 15 | 2440000 | 268 | 241 | 15 | 923000 |
| 8 | Tefluthrin | 177.1 | 127 | 15 | 18800000 | 197.1 | 141 | 10 | 6350000 |
| 9 | Tolcloflos-methyl | 265 | 250 | 15 | 14300000 | 267 | 252 | 10 | 5550000 |
| 10 | Fenthion | 278 | 109 | 15 | 10100000 | 278 | 124 | 20 | 3870000 |
| 11 | Thiamethoxam | 212 | 139 | 10 | 3170000 | 246.9 | 182 | 10 | 1680000 |
| 12 | Fosthiazate 1 | 195 | 103 | 5 | 1460000 | 283 | 103 | 15 | 303000 |
| 13 | Fosthiazate 2 | 195 | 103 | 5 | 1460000 | 283 | 103 | 15 | 303000 |
| 14 | Fipronil-sulfide | 419.9 | 351 | 10 | 8720000 | 350.9 | 255 | 15 | 12100000 |
| 15 | Fipronil | 367 | 213 | 20 | 2950000 | 367 | 255 | 15 | 1110000 |
| 16 | Formatanate | 163 | 122 | 10 | 415000 | 122 | 77 | 10 | 124000 |
| 17 | Paclobutrazole | 236 | 125 | 15 | 1430000 | 238 | 127 | 15 | 564000 |
| 18 | Mepanipyrim | 222.1 | 207 | 15 | 3590000 | 223.1 | 207 | 20 | 1880000 |
| 19 | Fenamiphos | 154 | 139 | 10 | 1310000 | 303 | 195 | 5 | 2000000 |
| 20 | Fipronil sulfone | 382.9 | 255 | 20 | 2270000 | 255 | 228 | 10 | 660000 |
| 21 | Chlorfenapyr | 327.9 | 247 | 15 | 536000 | 363.9 | 248 | 20 | 142000 |
| 22 | Fenthionsulfoxid | 277.9 | 109 | 15 | 1480000 | 246.9 | 125 | 10 | 856000 |
| 23 | Fenthionsulfon | 309.9 | 105 | 10 | 4010000 | 136.1 | 91 | 20 | 481000 |
| 24 | Triazophos | 257 | 162 | 5 | 11200000 | 285 | 162 | 10 | 10600000 |
| 25 | Fenamiphos sulfoxide | 304 | 122 | 15 | 3380000 | 288.0 | 243.0 | 5.0 | 1880000 |
| 26 | Fenamiphos sulphone | 320 | 292 | 10 | 11700000 | 291.9 | 134 | 15 | 3350000 |
| 27 | Phosmet | 160 | 77 | 20 | 1820000 | 160 | 133 | 10 | 1300000 |
| 28 | Amitraz | 162.2 | 132 | 10 | 3090000 | 132.1 | 117 | 10 | 3130000 |

| GC-MS/MS | | Parent 1 | Fragment 1 | Col. energi 1 | Intensity | Parent 2 | Fragment 2 | Col. energi 2 | Intensity |
|---|---|---|---|---|---|---|---|---|---|
| 29 | Boscalid | 341.9 | 140 | 15 | 6840000 | 167 | 139 | 20 | 295000 |
| 30 | Etofenprox | 163 | 107 | 15 | 14700000 | 107 | 77 | 15 | 1340000 |
| 31 | Indoxacarb | 264 | 176 | 10 | 922000 | 527 | 264 | 5 | 427000 |
| 32 | Propagite 1 | 135 | 107 | 5 | 1380000 | 350.2 | 81 | 10 | 300000 |
| 33 | Propagite 2 | 135 | 107 | 5 | 1380000 | 350.2 | 81 | 10 | 300000 |
| 34 | Bromuconazole 1 | 295 | 173 | 10 | 1260000 | 293 | 173 | 15 | 1010000 |
| 35 | Bromuconazole 2 | 295 | 173 | 10 | 1260000 | 293 | 173 | 15 | 1010000 |

| LC-MS/MS ESI- | | Moder ion | CV | Daughter-1 | CE | Daughter-2 | CE |
|---|---|---|---|---|---|---|---|
| 1 | Clothianidin | 247.923 | 50 | 58.134 | 13 | 150 | 13 |
| 2 | Methoxyfenozide | 367.43 | 42 | 149.001 | 23 | 105.114 | 31 |
| 3 | Tebufenozide | 351.43 | 42 | 149.035 | 23 | 105.044 | 31 |
| 4 | Fipronil | 435.22 | 42 | 330.152 | 13 | 250.069 | 23 |
| 5 | Fipronil-sulfide | 419.215 | 42 | 261.999 | 13 | 383.161 | 23 |
| 6 | Fipronil sulfone | 451.18 | 42 | 282.066 | 31 | 244.064 | 40 |
| 7 | Prothioconazole | 342.176 | 20 | 99.932 | 23 | 124.996 | 23 |
| 8 | Lufenuron | 509.216 | 13 | 339.186 | 13 | 175.009 | 31 |
| 9 | Diafenthiurone | 383.916 | 50 | 191.432 | 30 | 268.677 | 25 |
| 10 | Teflubenzuron | 379.176 | 42 | 339.107 | 23 | 195.953 | 13 |
| 11 | Dinocap | 295.1 | 20 | 209.1 | 20 | 192.1 | 20 |

| LCMSMS ESI+ | | Moder ion | CV | Daughter-1 | CE | Daughter-2 | CE |
|---|---|---|---|---|---|---|---|
| 1 | Formetanate | 222.226 | 30 | 165.04 | 20 | 92.92 | 20 |
| 2 | Thiamethoxam | 292.256 | 50 | 211.21 | 13 | 181.169 | 23 |
| 3 | 2.4 dimethylphenyl-N-methylformamidine | 163.715 | 10 | 122.955 | 20 | 132.936 | 20 |
| 4 | Imidacloprid | 256.41 | 30 | 175.27 | 13 | 209.39 | 13 |
| 5 | Thiacloprid | 253.204 | 10 | 126.115 | 23 | 90.287 | 40 |
| 6 | N-2,4-dimethylphenylformamide | 150.25 | 34 | 106.827 | 35 | 132.213 | 20 |
| 7 | Fenamiphos sulfoxide | 320.365 | 50 | 108.133 | 13 | 292.323 | 40 |
| 8 | Fenamiphos sulphone | 353.441 | 50 | 188.224 | 31 | 266.266 | 23 |
| 9 | Fenthionsulfoxid | 295.1 | 25 | 108.9 | 20 | 279.7 | 15 |
| 10 | Fenthionsulfon | 311.1 | 25 | 125 | 20 | 108.9 | 20 |
| 11 | Fosthiazate | 284.355 | 30 | 104.093 | 25 | 228.145 | 13 |

| LCMSMS ESI+ | | Moder ion | CV | Daughter-1 | CE | Daughter-2 | CE |
|---|---|---|---|---|---|---|---|
| 12 | Phosmet | 335.314 | 20 | 161.202 | 31 | 160 | 39 |
| 13 | Fenamidon | 312.484 | 30 | 236.329 | 40 | 92.117 | 13 |
| 14 | Boscalid | 343.35 | 57 | 307.22 | 20 | 140.25 | 20 |
| 15 | Paclobutrazole | 294.331 | 20 | 70.23 | 31 | 125.153 | 31 |
| 16 | Triazophos | 314.312 | 42 | 162.236 | 13 | 119.193 | 40 |
| 17 | Ethoprophos | 243.241 | 50 | 97.049 | 23 | 131.056 | 31 |
| 18 | Diphenylamine | 170 | 25 | 93.1 | 20 | 151.9 | 20 |
| 19 | Mepanipyrim | 224.204 | 42 | 77.196 | 23 | 106.17 | 39 |
| 20 | Fenamiphos | 304.41 | 50 | 217.079 | 23 | 202.069 | 31 |
| 21 | Prothioconazole desthio | 312.484 | 50 | 70.34 | 35 | 125.424 | 30 |
| 22 | Fenoxycarb | 302.331 | 42 | 88.215 | 31 | 116.228 | 13 |
| 23 | Fenthion | 279.1 | 25 | 169.1 | 20 | 247.1 | 20 |
| 24 | Bromuconazole 1 | 378.232 | 42 | 159.072 | 23 | 70.182 | 23 |
| 25 | Bromuconazole 2 | 378.232 | 42 | 159.072 | 23 | 70.182 | 23 |
| 26 | Zoxamide | 336.39 | 36 | 159.125 | 20 | 187.19 | 40 |
| 27 | Phoxim | 299.298 | 10 | 115.029 | 31 | 77.1 | 23 |
| 28 | Indoxacarb | 528.458 | 42 | 203.494 | 31 | 150.34 | 31 |
| 29 | Tolcloflos-methyl | 301 | 25 | 175 | 20 | 268.9 | 20 |
| 30 | Clofentezin | 303.1 | 20 | 138.1 | 20 | 102.1 | 20 |
| 31 | Pencycuron | 329.458 | 50 | 125.123 | 23 | 218 | 13 |
| 32 | Spinosad A | 733.01 | 30 | 142.182 | 20 | 98 | 20 |
| 33 | Cadusafos | 271.331 | 50 | 159.025 | 13 | 97.018 | 40 |
| 34 | Spinosad D | 746.5 | 30 | 142.311 | 40 | 98.168 | 45 |
| 35 | Flufenoxuron | 489.614 | 22 | 158.147 | 13 | 140.949 | 40 |
| 36 | Propagite | 368.478 | 30 | 175.231 | 13 | 231.406 | 13 |
| 37 | Pyrethrins | 329.503 | 34 | 128.211 | 31 | 162.138 | 13 |
| 38 | Amitraz | 294.486 | 30 | 163.181 | 13 | 122.104 | 31 |
| 39 | Avermectin B | 890.5 | 41 | 145.2 | 43 | 305.1 | 35 |
| 40 | Etofenprox | 394.2 | 25 | 177.3 | 20 | 107.1 | 20 |

# Appendix 2. Repeatability, recovery and limit of quantification for compounds validated on LC-MS-MS.

In the tables are presented repeatability and LOQ for compounds validated on LC-MS-MS. Values outside the acceptance criteria are marked by grey. The matrixes are wheat, oat and rye.

| **WHEAT** | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.011 | 32 | | 0.02 | 29 | | 0.104 | 22 | | |
| | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **LOQ** |
| Clothianidin | 70 | 26 | | 103 | 3 | | 95 | 3 | | 0.012 |
| Methoxyfenozide | 59 | 30 | | 87 | 11 | | 91 | 11 | | 0.011 |
| Tebufenozide | 64 | 8 | | 91 | 8 | | 101 | 7 | | 0.009 |
| Fipronil | 69 | 11 | | 87 | 11 | | 91 | 8 | | 0.009 |
| Fipronil-sulfide | 80 | 28 | | 96 | 12 | | 98 | 9 | | 0.015 |
| Fipronil sulfone | 65 | 36 | | 96 | 6 | | 102 | 8 | | 0.007 |
| Lufenuron | 57 | 13 | | 92 | 9 | | 86 | 6 | | 0.009 |
| Teflubenzuron | 53 | 30 | | 79 | 11 | | 90 | 10 | | 0.010 |
| Formetanate | 88 | 21 | | 84 | 19 | | 87 | 3 | | 0.012 |
| Thiamethoxam | 127 | 57 | | 109 | 36 | | 86 | 8 | | 0.041 |
| Thiacloprid | 93 | 13 | | 92 | 9 | | 86 | 6 | | 0.008 |
| Avermectin B | 99 | 62 | | 113 | 63 | | 96 | 22 | | 0.133 |
| Etofenprox | 70 | 13 | | 69 | 20 | | 78 | 12 | | 0.006 |
| Fenamiphos sulphone | 87 | 18 | | 88 | 26 | | 15 | 15 | | 0.011 |
| Fenthionsulfoxid | 98 | 44 | | 105 | 3 | | 100 | 7 | | 0.029 |
| Fenthionsulfon | 104 | 27 | | 124 | 40 | | 97 | 21 | | 0.126 |
| Fosthiazate | 84 | 21 | | 85 | 9 | | 73 | 10 | | 0.012 |
| Phosmet | 88 | 12 | | 94 | 14 | | 96 | 10 | | 0.007 |
| Boscalid | 102 | 35 | | 75 | 26 | | 94 | 15 | | 0.023 |
| Paclobutrazole | 83 | 31 | | 98 | 5 | | 106 | 8 | | 0.017 |
| Triazophos | 80 | 20 | | 80 | 12 | | 77 | 12 | | 0.010 |
| Ethoprophos | 79 | 19 | | 102 | 9 | | 99 | 9 | | 0.010 |
| Mepanipyrim | 114 | 64 | | 77 | 108 | | 102 | 19 | | 0.118 |
| Fenamiphos | 85 | 17 | | 82 | 17 | | 74 | 8 | | 0.010 |
| Fenoxycarb | 106 | 4 | | 98 | 21 | | 79 | 7 | | 0.003 |
| Bromuconazole sum | 112 | 22 | | 90 | 10 | | 76 | 15 | | 0.016 |
| Phoxim | 95 | 18 | | 82 | 23 | | 95 | 18 | | 0.011 |
| Propagite | 89 | 14 | | 98 | 19 | | 90 | 11 | | 0.008 |
| Clofentezin | 52 | 99 | | 122 | 79 | | 76 | 10 | | 0.047 |
| Pencycuron | 87 | 27 | | 81 | 17 | | 80 | 10 | | 0.015 |
| Spinosad A | 81 | 23 | | 100 | 16 | | 81 | 10 | | 0.013 |
| Cadusafos | 91 | 15 | | 97 | 6 | | 85 | 10 | | 0.009 |

| OAT | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.011 | 32 | | 0.02 | 29 | | 0.104 | 22 | | |
| | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **LOQ** |
| Clothianidin | 89 | 15 | | 109 | 17 | | 95 | 17 | | 0.009 |
| Methoxyfenozide | 88 | 16 | | 98 | 18 | | 103 | 18 | | 0.009 |
| Tebufenozide | 69 | 20 | | 97 | 20 | | 97 | 6 | | 0.023 |
| Fipronil | 81 | 18 | | 98 | 18 | | 103 | 5 | | 0.015 |
| Fipronil-sulfide | 85 | 29 | | 91 | 16 | | 91 | 9 | | 0.016 |
| Fipronil sulfone | 87 | 21 | | 94 | 14 | | 91 | 6 | | 0.012 |
| Lufenuron | 68 | 14 | | 120 | 13 | | 102 | 7 | | 0.018 |
| Teflubenzuron | 71 | 18 | | 77 | 16 | | 78 | 3 | | 0.008 |
| Formetanate | 84 | 20 | | 92 | 10 | | 83 | 9 | | 0.011 |
| Thiamethoxam | 96 | 30 | | 111 | 17 | | 82 | 19 | | 0.019 |
| Thiacloprid | 95 | 14 | | 120 | 13 | | 102 | 7 | | 0.009 |
| Avermectin B | 60 | 29 | | 83 | 18 | | 94 | 22 | | 0.018 |
| Etofenprox | 71 | 16 | | 86 | 29 | | 93 | 13 | | 0.007 |
| Fenamiphos sulphone | 93 | 14 | | 111 | 5 | | 111 | 6 | | 0.008 |
| Fosthiazate | 92 | 11 | | 118 | 5 | | 108 | 8 | | 0.007 |
| Phosmet | 93 | 16 | | 107 | 4 | | 105 | 6 | | 0.010 |
| Boscalid | 85 | 20 | | 108 | 9 | | 108 | 6 | | 0.011 |
| Paclobutrazole | 93 | 20 | | 117 | 8 | | 108 | 7 | | 0.012 |
| Triazophos | 95 | 12 | | 99 | 19 | | 104 | 6 | | 0.007 |
| Ethoprophos | 88 | 16 | | 99 | 11 | | 101 | 8 | | 0.009 |
| Diphenylamine | 53 | 51 | | 59 | 51 | | 104 | 6 | | 0.040 |
| Mepanipyrim | 100 | 14 | | 99 | 17 | | 101 | 8 | | 0.009 |
| Fenamiphos | 87 | 11 | | 103 | 7 | | 100 | 8 | | 0.006 |
| Fenoxycarb | 79 | 16 | | 104 | 6 | | 106 | 6 | | 0.008 |
| Bromuconazole sum | 82 | 10 | | 107 | 10 | | 107 | 5 | | 0.005 |
| Zoxamide | 947 | 204 | | 97 | 22 | | 97 | 11 | | 0.025 |
| Phoxim | 2092 | 179 | | 89 | 22 | | 96 | 10 | | 0.023 |
| Propagite | 86 | 15 | | 99 | 15 | | 106 | 7 | | 0.009 |
| Tolcloflos-methyl | 972 | 206 | | 87 | 34 | | 97 | 18 | | 0.111 |
| Clofentezin | 273 | 146 | | 107 | 14 | | 100 | 20 | | 0.019 |
| Pencycuron | 97 | 9 | | 108 | 4 | | 105 | 6 | | 0.005 |
| Spinosad A | 75 | 16 | | 87 | 19 | | 102 | 9 | | 0.008 |
| Cadusafos | 84 | 10 | | 103 | 7 | | 100 | 10 | | 0.006 |
| Spinosad D | 83 | 32 | | 105 | 15 | | 105 | 8 | | 0.018 |

| RYE | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.011 | 32 | | 0.02 | 29 | | 0.104 | 22 | | |
| | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **LOQ** |
| Clothianidin | 89 | 18 | | 109 | 7 | | 97 | 11 | | 0.011 |
| Methoxyfenozide | 77 | 16 | | 97 | 12 | | 90 | 13 | | 0.008 |
| Tebufenozide | 73 | 34 | | 92 | 10 | | 93 | 9 | | 0.011 |
| Fipronil | 87 | 21 | | 97 | 12 | | 90 | 13 | | 0.012 |
| Fipronil-sulfide | 77 | 19 | | 107 | 8 | | 102 | 9 | | 0.010 |
| Fipronil sulfone | 84 | 12 | | 103 | 4 | | 96 | 12 | | 0.007 |
| Lufenuron | 81 | 17 | | 80 | 5 | | 81 | 11 | | 0.009 |
| Teflubenzuron | 112 | 17 | | 76 | 14 | | 76 | 13 | | 0.012 |
| Formetanate | 89 | 15 | | 110 | 8 | | 103 | 9 | | 0.009 |
| Thiamethoxam | 55 | 36 | | 82 | 18 | | 106 | 9 | | 0.018 |
| Thiacloprid | 81 | 18 | | 101 | 9 | | 104 | 9 | | 0.010 |
| Etofenprox | 70 | 34 | | 79 | 16 | | 80 | 9 | | 0.015 |
| Fenamiphos sulphone | 82 | 25 | | 105 | 10 | | 110 | 6 | | 0.014 |
| Fenthionsulfoxid | 63 | 37 | | 88 | 19 | | 106 | 10 | | 0.019 |
| Fosthiazate | 77 | 11 | | 106 | 12 | | 111 | 7 | | 0.005 |
| Phosmet | 80 | 20 | | 102 | 5 | | 104 | 6 | | 0.010 |
| Boscalid | 91 | 38 | | 102 | 10 | | 110 | 8 | | 0.012 |
| Paclobutrazole | 69 | 18 | | 110 | 6 | | 110 | 9 | | 0.008 |
| Triazophos | 62 | 23 | | 100 | 10 | | 90 | 13 | | 0.012 |
| Ethoprophos | 80 | 18 | | 112 | 14 | | 111 | 8 | | 0.009 |
| Diphenylamine | 57 | 116 | | 48 | 19 | | 90 | 13 | | 0.07 |
| Mepanipyrim | 73 | 26 | | 80 | 16 | | 82 | 19 | | 0.013 |
| Fenamiphos | 76 | 22 | | 103 | 6 | | 108 | 5 | | 0.011 |
| Fenoxycarb | 91 | 20 | | 100 | 11 | | 110 | 9 | | 0.012 |
| Bromuconazole sum | 91 | 15 | | 110 | 14 | | 108 | 7 | | 0.009 |
| Zoxamide | 60 | 84 | | 121 | 60 | | 117 | 21 | | 0.152 |
| Phoxim | 76 | 53 | | 100 | 23 | | 112 | 5 | | 0.027 |
| Propagite | 71 | 12 | | 91 | 20 | | 85 | 8 | | 0.005 |
| Tolcloflos-methyl | 64 | 170 | | 97 | 86 | | 93 | 4 | | 0.023 |
| Clofentezin | 109 | 50 | | 106 | 14 | | 108 | 7 | | 0.018 |
| Pencycuron | 78 | 8 | | 93 | 12 | | 92 | 8 | | 0.004 |
| Spinosad A | 70 | 22 | | 79 | 13 | | 89 | 5 | | 0.010 |
| Cadusafos | 70 | 19 | | 98 | 7 | | 106 | 4 | | 0.009 |
| Spinosad D | 52 | 56 | | 67 | 10 | | 84 | 10 | | 0.054 |

# Appendix 3. Repeatability, recovery and limit of quantification for compounds validated on GC-MS-MS.

| **WHEAT** | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.011 | 32 | | 0.02 | 29 | | 0.104 | 22 | | |
| | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **LOQ** |
| Clofentezin | 73 | 10 | | 71 | 7 | | 75 | 7 | | 0.005 |
| Diphenylamine | 96 | 11 | | 98 | 11 | | 70 | 12 | | 0.004 |
| Ethoprophos | 102 | 5 | | 103 | 5 | | 76 | 10 | | 0.012 |
| Pencycuron | 92 | 9 | | 88 | 7 | | 76 | 19 | | 0.005 |
| Cadusafos | 93 | 10 | | 105 | 3 | | 86 | 9 | | 0.006 |
| Flufenoxuron | 73 | 16 | | 80 | 13 | | 67 | 11 | | 0.008 |
| Tefluthrin | 93 | 16 | | 103 | 8 | | 109 | 6 | | 0.011 |
| Tolclofos-methyl | 98 | 11 | | 102 | 6 | | 108 | 9 | | 0.007 |
| Fenthion | 96 | 14 | | 99 | 4 | | 98 | 8 | | 0.009 |
| Thiamethoxam | 97 | 12 | | 103 | 8 | | 101 | 9 | | 0.008 |
| Fosthiazate sum | 101 | 3 | | 111 | 5 | | 111 | 2 | | 0.002 |
| Fipronil-sulfide | 93 | 18 | | 103 | 5 | | 103 | 8 | | 0.011 |
| Fipronil | 102 | 16 | | 103 | 8 | | 109 | 6 | | 0.011 |
| Paclobutrazole | 100 | 16 | | 110 | 5 | | 102 | 9 | | 0.010 |
| Mepanipyrim | 95 | 7 | | 100 | 7 | | 105 | 12 | | 0.004 |
| Fenamiphos | 89 | 10 | | 94 | 8 | | 82 | 19 | | 0.006 |
| Fipronil-sulfone | 92 | 11 | | 101 | 5 | | 110 | 4 | | 0.007 |
| Chlorfenapyr | 97 | 26 | | 91 | 13 | | 107 | 5 | | 0.017 |
| Fenthion-sulfoxid | 85 | 18 | | 92 | 6 | | 107 | 10 | | 0.010 |
| Fenthion-sulfon | 84 | 18 | | 96 | 4 | | 108 | 4 | | 0.010 |
| Triazophos | 94 | 11 | | 100 | 7 | | 113 | 1 | | 0.007 |
| Fenamiphos-sulfon | 97 | 15 | | 96 | 8 | | 100 | 5 | | 0.010 |
| Bromuconazole sum | 85 | 17 | | 100 | 9 | | 105 | 12 | | 0.009 |
| Phosmet | 102 | 9 | | 90 | 9 | | 74 | 14 | | 0.006 |
| Boscalid | 87 | 14 | | 91 | 6 | | 101 | 7 | | 0.008 |
| Etofenprox | 85 | 0 | | 81 | 6 | | 73 | 19 | | 0.005 |
| Indoxacarb | 85 | 8 | | 101 | 10 | | 106 | 6 | | 0.005 |

| **OAT** | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | Spike level mg/kg | Horwitz, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.011 | 32 | | 0.02 | 29 | | 0.104 | 22 | | |
| | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **Recovery, %** | **RSDr, %** | | **LOQ** |
| Clofentezin | 105 | 15 | | 89 | 9 | | 102 | 9 | | 0.011 |
| Lufenuron | 101 | 5 | | 71 | 4 | | 70 | 4 | | 0.003 |
| Ethoprophos | 115 | 3 | | 120 | 3 | | 81 | 7 | | 0.010 |
| Pencycuron | 78 | 10 | | 77 | 6 | | 82 | 6 | | 0.005 |
| Cadusafos | 119 | 12 | | 119 | 2 | | 82 | 6 | | 0.010 |
| Flufenoxuron | 86 | 15 | | 82 | 8 | | 70 | 6 | | 0.008 |
| Tefluthrin | 103 | 15 | | 110 | 4 | | 81 | 2 | | 0.012 |
| Tolclofos-methyl | 111 | 14 | | 119 | 5 | | 76 | 5 | | 0.010 |
| Fenthion | 85 | 16 | | 88 | 5 | | 70 | 7 | | 0.009 |
| Thiamethoxam | 112 | 8 | | 116 | 1 | | 76 | 3 | | 0.006 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Fipronil-sulfide | 118 | 14 | 119 | 8 | 88 | 3 | 0.011 |
| Fipronil | 126 | 15 | 131 | 4 | 81 | 2 | 0.011 |
| Paclobutrazole | 124 | 20 | 147 | 2 | 89 | 4 | 0.016 |
| Mepanipyrim | 111 | 31 | 116 | 7 | 70 | 5 | 0.023 |
| Fenamiphos | 99 | 12 | 110 | 3 | 74 | 3 | 0.008 |
| Fipronil-sulfone | 114 | 13 | 119 | 1 | 91 | 3 | 0.010 |
| Chlorfenapyr | 102 | 13 | 102 | 18 | 80 | 3 | 0.009 |
| Fenthion-sulfoxid | 132 | 18 | 144 | 4 | 84 | 13 | 0.068 |
| Fenthion-sulfon | 100 | 13 | 110 | 2 | 76 | 7 | 0.009 |
| Triazophos | 101 | 11 | 110 | 4 | 75 | 8 | 0.007 |
| Fenamiphos-sulfoxide | 105 | 9 | 106 | 3 | 72 | 8 | 0.006 |
| Fenamiphos-sulfon | 97 | 21 | 95 | 3 | 74 | 15 | 0.013 |
| Bromuconazole sum | 99 | 6 | 103 | 3 | 81 | 2 | 0.004 |
| Phosmet | 92 | 11 | 80 | 9 | 70 | 4 | 0.007 |
| Boscalid | 99 | 10 | 110 | 4 | 80 | 2 | 0.007 |
| Etofenprox | 95 | 0 | 102 | 5 | 73 | 4 | 0.010 |
| Indoxacarb | 91 | 11 | 92 | 2 | 77 | 17 | 0.006 |

| **RYE** | Spike level mg/kg | Horwitz, % | Spike level mg/kg | Horwitz, % | Spike level mg/kg | Horwitz, % | |
|---|---|---|---|---|---|---|---|
| | 0.011 | 32 | 0.02 | 29 | 0.104 | 22 | |
| | **Recovery, %** | **RSDr, %** | **Recovery, %** | **RSDr, %** | **Recovery, %** | **RSDr, %** | **LOQ** |
| Clofentezin | 92 | 18 | 103 | 9 | 99 | 9 | 0.011 |
| Diphenylamine | 107 | 7 | 110 | 7 | 81 | 3 | 0.009 |
| Ethoprophos | 98 | 6 | 118 | 6 | 89 | 4 | 0.008 |
| Pencycuron | 98 | 10 | 102 | 7 | 82 | 2 | 0.007 |
| Cadusafos | 96 | 12 | 117 | 2 | 84 | 2 | 0.007 |
| Flufenoxuron | 76 | 18 | 85 | 7 | 109 | 6 | 0.009 |
| Tefluthrin | 91 | 8 | 110 | 4 | 83 | 4 | 0.005 |
| Tolclofos-methyl | 95 | 12 | 114 | 3 | 84 | 3 | 0.007 |
| Fenthion | 96 | 11 | 119 | 3 | 79 | 3 | 0.007 |
| Thiamethoxam | 85 | 15 | 100 | 8 | 79 | 7 | 0.008 |
| Fosthiazate sum | 99 | 12 | 108 | 7 | 97 | 31 | 0.008 |
| Fipronil-sulfide | 98 | 13 | 115 | 2 | 83 | 3 | 0.008 |
| Fipronil | 101 | 8 | 110 | 4 | 83 | 4 | 0.005 |
| Paclobutrazole | 100 | 11 | 119 | 5 | 80 | 6 | 0.007 |
| Mepanipyrim | 99 | 11 | 110 | 5 | 75 | 9 | 0.007 |
| Fenamiphos | 91 | 11 | 110 | 8 | 76 | 4 | 0.007 |
| Fipronil-sulfone | 113 | 22 | 119 | 2 | 99 | 5 | 0.016 |
| Chlorfenapyr | 95 | 14 | 116 | 3 | 81 | 2 | 0.009 |
| Fenthion-sulfoxid | 94 | 13 | 88 | 3 | 77 | 12 | 0.008 |
| Fenthion-sulfon | 97 | 13 | 102 | 4 | 83 | 4 | 0.008 |
| Triazophos | 87 | 16 | 109 | 2 | 78 | 9 | 0.009 |
| Fenamiphos-sulfoxide | 100 | 7 | 89 | 10 | 87 | 16 | 0.005 |
| Fenamiphos-sulfon | 95 | 11 | 94 | 5 | 87 | 22 | 0.007 |
| Bromuconazole sum | 98 | 12 | 110 | 3 | 78 | 2 | 0.007 |
| Phosmet | 96 | 20 | 90 | 8 | 83 | 5 | 0.013 |
| Boscalid | 93 | 13 | 109 | 6 | 80 | 4 | 0.008 |
| Etofenprox | 90 | 0 | 107 | 8 | 74 | 2 | 0.008 |
| Indoxacarb | 87 | 11 | 95 | 8 | 275 | 63 | 0.006 |