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National Food Institute
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Validation Report 17

Determination of pesticide residues in maize for livestock feed by GC-MS/MS and LC-MS/MS

(QuEChERS method)

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CONTENT:

1. Introduction *3*
2. Principle of analysis *3*
3. Validation design *4*
4. Chromatograms and calibration curves *4*
5. Validation parameters *6*
6. Criteria for the acceptance of validation results *7*
7. Results and discussion *7*
8. Conclusions *7*
9. References *7*
Appendix 1a. MRM transitions GC-MS/MS. *9*
Appendix 1b. MRM transitions for LC-MS/MS. *9*
Appendix 2. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQ) for pesticides validated on maize for livestock feed. *10*
Appendix 3: Principles of the QuEChERS method for feed extraction *11*

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 9 pesticides in a feed variety of maize. The QuEChERS method is an extraction method which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit, vegetables and cereals[1].

2. Principle of analysis

Sample preparation: The samples is milled with a sieve at 1 mm.

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 clean tube and put in -80 degree freezer. When the extract is almost thawed it is centrifuged and the supernatant is transferred to a tube containing PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract is diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the matrix matched calibration standards.

The extraction and clean-up procedure is outlined in **Appendix 3**.

Quantification and qualification: The final extract is analysed by GC/MS/MS and LC-MS/MS.

GC-MS/MS: The pesticide residues are separated on a DB5-MS column and analysed by triple quadrupole operating in the multiple reaction monitoring mode (MRM) with electron energy at 70 eV, source temperature at 180°C and transfer line at 250°C. The injection volume was 4 µl. For each pesticide two sets of precursor and product ions were determined. One for quantification and one for qualification. The MRM transitions for the pesticides and degradation products are given in **Appendix 1a**.

LC-MS/MS: The pesticide residues are separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI). The validation includes pesticides determined with both positive and negative ESI. $^{13}C_6$-carbaryl was used as internal standard but was not used for the quantification. All pesticides were detected in the MRM mode. For each pesticide precursor ion and 2 product ions 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 1b**.

3. Validation design

The method was south validated for 9 pesticides or degradation products in maize see **Table 1**. The validation was performed on 5-6 replicates on each of the four spiking levels; 0.01, 0.02, 0.04 and 0.2 mg/kg. A blank sample of each maize was included.

Table 1. Pesticides included in the recovery experiments.

Pesticides included in recovery experiments	
Cypermethrin	Propiconazole
Epoxiconazole	Prothioconazole-desthio
Lambda-cyhalothrin	Pyraclostrobin
Pendimethalin	Tebuconazole
Prochloraz	

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 and 0.1 µg/ml. The calibration curves were in general best fitted to a linear curve. The quantification was performed from the mean of two bracketing calibration curves. The majority of the correlation coefficients (R) were higher or equal to 0.99. Examples of chromatograms and calibration curves obtained when analysing extracts by GC-MS/MS and LC-MS/MS are presented in **Figure 1-2** and **Figure 3-4**, respectively.

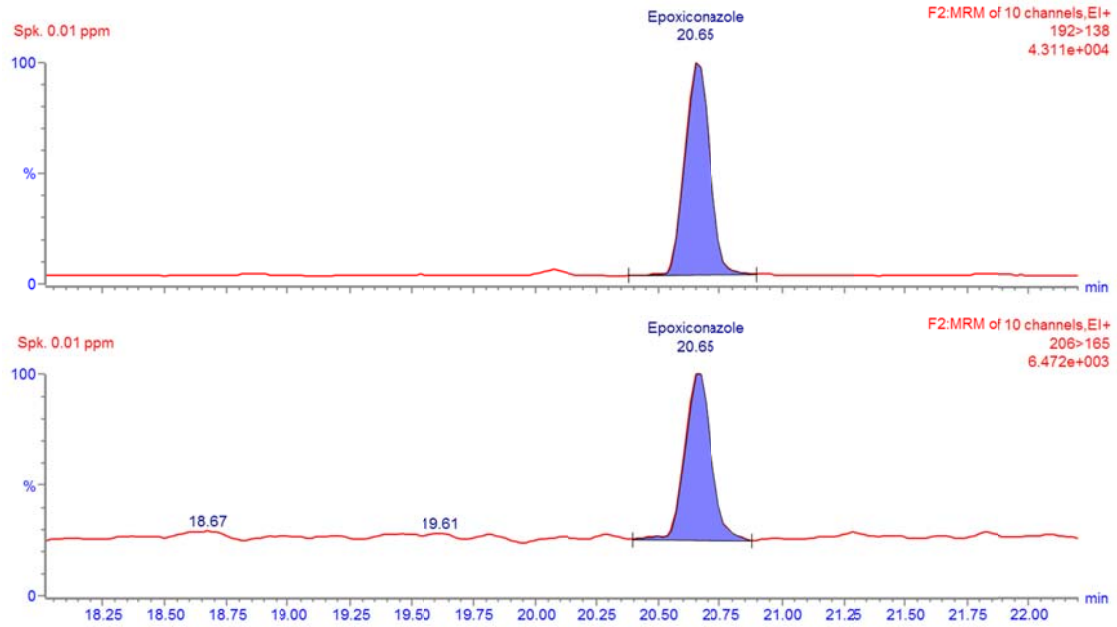

Figure 1: Examples of GC-MS/MS chromatograms for epoxiconazole in maize for livestock feed obtained when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown).

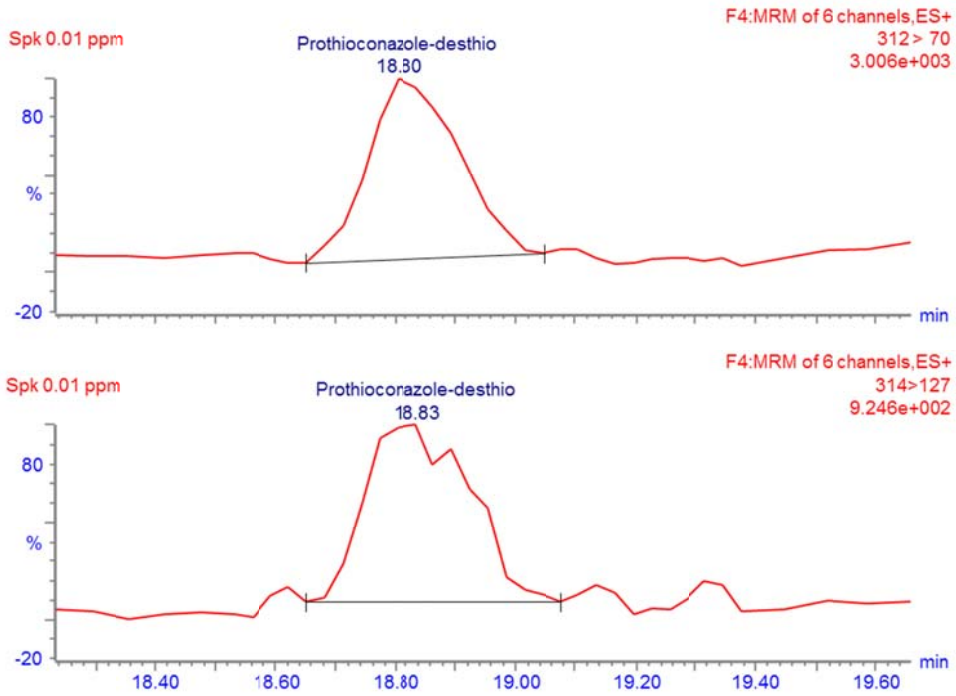

Figure 2: Examples of LC-MS/MS chromatograms Prothioconazole-desthio in maize for livestock feed obtained in positive mode when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown).

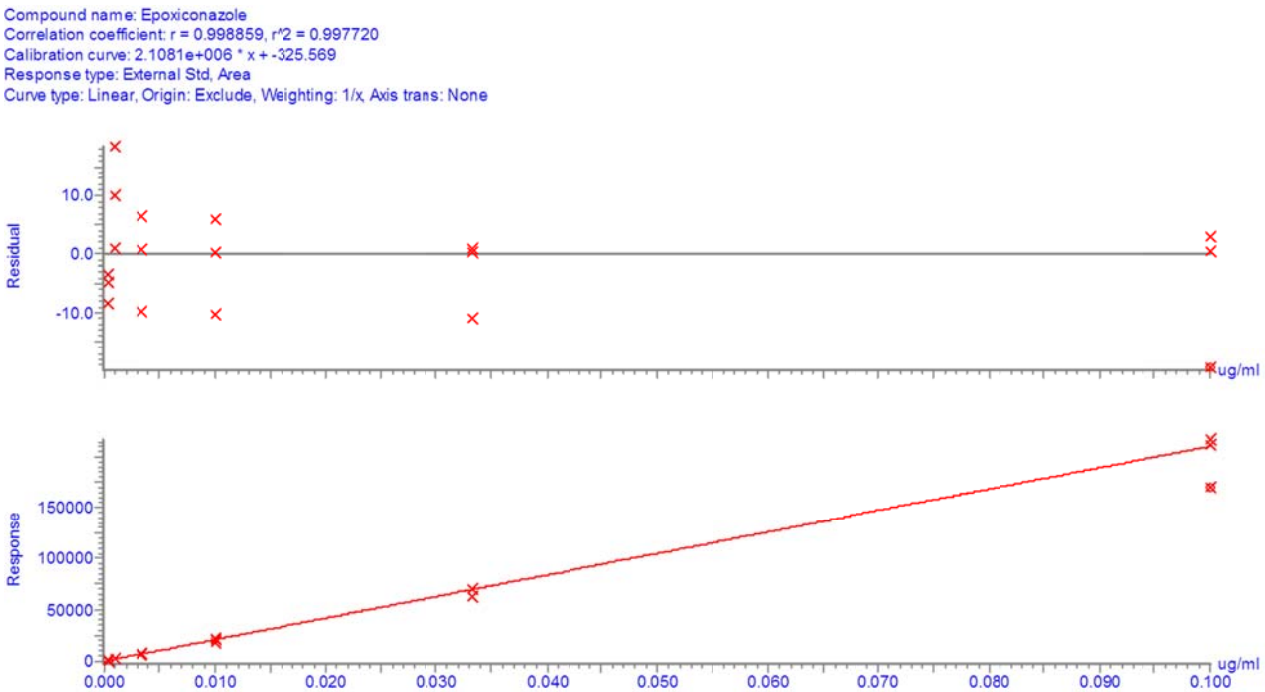

Figure 3. Examples of GC-MS/MS calibration curves for epoxiconazole matrix matched with maize for livestock feed (concentrations from 0.0003-0.1 µg/ml)

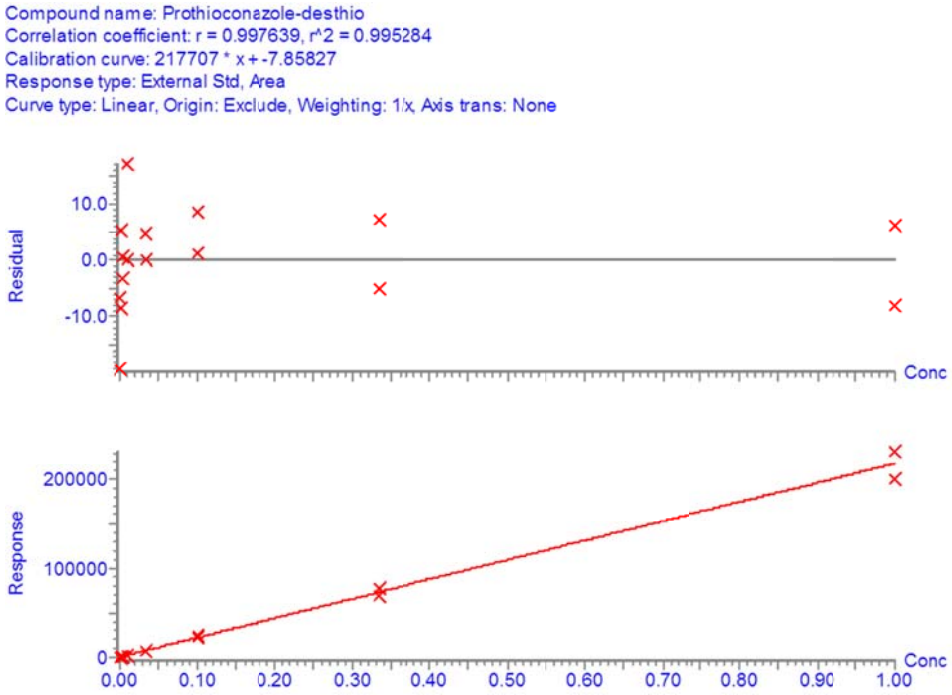

Figure 4. Examples of LC-MS/MS calibration curves for prothioconazole-dethio matrix matched with maize for livestock feed (concentrations from 0.0003-1.0 µg/ml).

5. Validation parameters

Precision – repeatability

Repeatability was calculated for all pesticides and degradation products on four spiking levels (0.01, 0.02, 0.04 and 0.2 mg/kg). 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 (RSD_r) in this validation was calculated from the 5-6 replicate determinations as given in ISO 5725-2[2].

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.01, 0.02, 0.04 and 0.2 mg/kg) with the relevant pesticides, isomers and degradation products.

Robustness

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

Limit of quantification, LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria (se Section 6) was meet.

The obtained results including recovery, RSD_r and limit of quantification (LOQ), are presented in appendix 2.

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 should be $\leq 20\%$[3].
2. The average relative recovery must be between 70 and 120%[3].

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

7. Results and discussion

Overall validation

All 9 compounds included in the validation study (Table 1) were successfully validated for maize on GC-MS/MS (2 pesticides), LC-MS/MS (2 pesticides) or both (5 pesticides), see **Appendix 2**.

For the accepted validation parameters the relative repeatability (RSD_r) varied between 2-14 % with an average of 7%. Recoveries were in the range of 75-118% at all concentration levels with an average of 95%. LOQs were for all analytes 0.01 mg/kg.

8. Conclusions

In conclusion 9 pesticides were successfully validated on maize for livestock feeding using the QuEChERS method and GC-MS/MS or/and LC-MS/MS. The LOQ obtained were 0.01 mg/kg.

9. 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 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

3 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/12495/2011, 01/01/2012, European Commission, Brussels, 2012.

Appendix 1a. MRM transitions GC-MS/MS.

GC-MS/MS	Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CE
Cypermethrin	27	163	127	10	181	152	20
Epoxiconazole	21.34	192	138	10	206	165	5
Lambda-Cyhalothrin-1	23.64	197	141	10	208	181	10
Lambda-Cyhalothrin-2	24	197	141	10	208	181	10
Pendimethalin	15.6	281	252	5	252	162	5
Prochloraz	26	180	138	10	310	268	5
Propiconazole-1	20	173	145	15	259	173	15
Propiconazole-2	20	173	145	15	259	173	15
Tebuconazole	20.7	250	125	15	125	89	10

Appendix 1b. MRM transitions for LC-MS/MS.

LC-MS/MS	Retention time	Precursor ion-1	Product ion-1	CV	CE	Precursor ion-2	Product ion-2	CV	CE
Epoxiconazole	18.6	330	121	45	23	330	91	45	41
Pendimethalin	23.55	282	212	33	10	282	194	33	10
Prochloraz	20.45	376	308	20	10	378	310	31	10
Propiconazole	19.77	342	159	20	20	342	69	20	20
Prothioconazole_desthio	19.72	312	70	50	35	314	127	50	35
Pyraclostrobin	20.17	388	194	24	11	388	163	24	25
Tebuconazole	19.7	308	70	20	20	310	70	20	20

Appendix 2. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQ) for pesticides validated on maize for livestock feed.

		Spike level mg/kg 0.01			Spike level mg/kg 0.02			Spike level mg/kg 0.04			Spike level mg/kg 0.2		
		Recovery, %	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**	**LOQ**
GC	Cypermethrin (sum of isomers)	97	9		99	9		86	9		95	6	0.01
GC	Epoxiconazole	105	4		108	4		89	6		100	4	0.01
LC	Epoxiconazole	98	3		97	7		100	9		96	5	0.01
GC	Lambda-cyhalothrin	105	10		101	8		88	7		97	5	0.01
GC	Pendimethalin	93	2		94	5		83	9		93	5	0.01
LC	Pendimethalin	75	14		78	8		86	9		91	6	0.01
GC	Prochloraz	95	7		98	6		83	10		96	7	0.01
LC	Prochloraz	86	14		95	5		103	10		103	6	0.01
LC	Propiconazole	94	9		96	5		96	8		92	7	0.01
GC	Propiconazole	118	14		108	6		95	6		101	4	0.01
LC	Prothioconazole-desthio	97	8		89	5		90	10		86	4	0.01
LC	Pyraclostrobin	87	10		94	5		103	9		100	5	0.01
GC	Tebuconazole	97	7		99	4		82	7		93	4	0.01
LC	Tebuconazole	98	10		95	4		94	8		90	4	0.01

Appendix 3: Principles of the QuEChERS method for feed extraction

QuEChERS for cereals (FP417)

Weigh 5 g (±0.05 g) of flour into a 50 ml single use centrifuge tube (red cap). Add internal standard and/or spike standard (maximum 25 µl)

Add a ceramic homogenizer and 10 g of cold water and shake briefly

Add 10 ml acetonitrile and shake vigorously by hand for 1 min. (1. extraction)

Add the prepared mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3 citrate dihydrate and 0.5 g Na_2H cirate sesquihydrate. Shake for a few seconds after each addition to prevent lumps.

Shake vigorously for 1 min. (2. Extraction with phase separation)

Centrifuge for 10 min at 4500 rpm

Transfer at least 8 ml of the extract to a 15 ml single use centrifuge tube and store in the freezer (-80˚C for 1 hour or over night). When the extract are almost thawed (i.e. About -40 ˚C) centrifugate (should be cold 5 C) for 5 min. at 4500 rpm.

Transfer 6 ml of the cold extract to a 15 ml single use centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$. Close the tube and shake vigorously for 30 seconds.

Centrifuge for 5 min. at 4500 rpm

Transfer 4 ml of the extract to a 15 ml single use centrifuge tube. Add 40 µl of 5% formic acid solution in acetonitrile (10 µl/ml extract). Dilute the extract 1:1 with acetonitrile

Transfer the final extract into auto sampler vials and analyse by GC and LC.