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National Food Institute
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Validation Report 18

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

This report describes the validation of the QuEChERS method combined with GC-MS/MS and LC-MS/MS. The method was sought validated for 11 pesticides in maize for livestock feed. 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 principle of 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 11 pesticides or degradation products in maize see **Table 1**. The validation was performed on 5-6 replicates at each of four spiking levels; 0.005, 0.01, 0.02 and 0.1 mg/kg. A blank sample of each maize was included.

Table 1. Pesticides included in the recovery experiments.

Pesticides included in recovery experiments	
Bifenazate	Spirotetramat
Fluopyram	Spirotetramat-enol
Foramsulfuron	Spirotetramat-glu
Iodosulfuron-methyl	Spirotetramat-keto
Isoxadifen-ethyl	Spirotetramat-mono
Spiromesifen	

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.

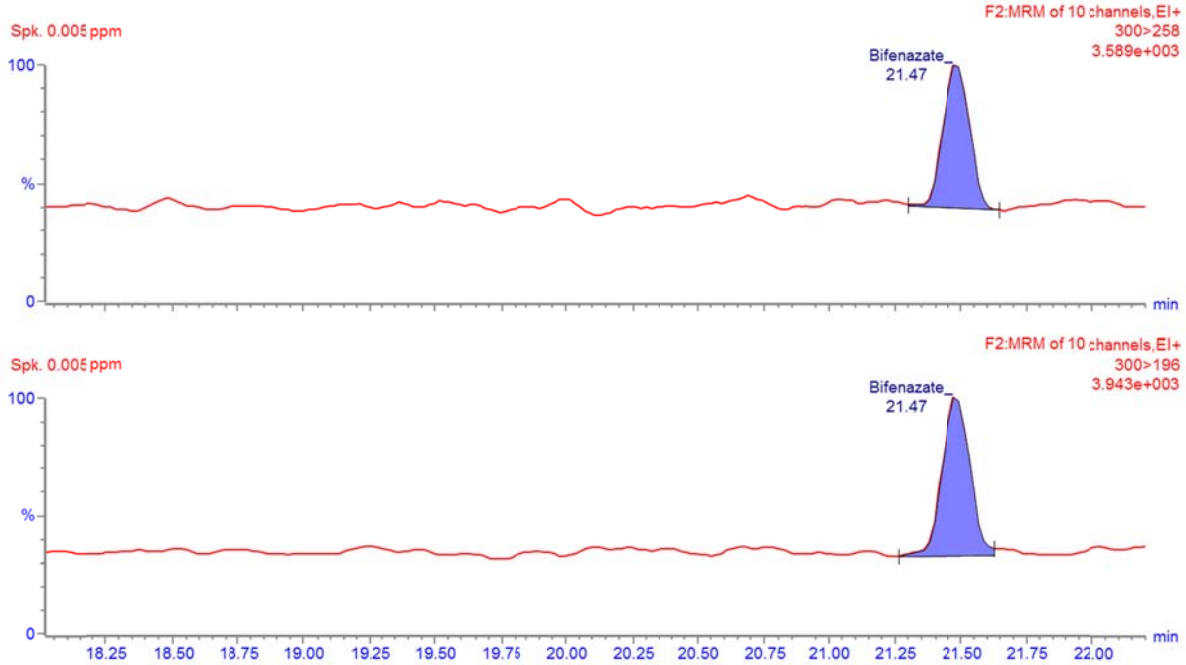

Figure 1: Examples of GC-MS/MS chromatograms for bifenazate in maize for livestock feed obtained when analysing extract spiked with 0.005 mg/kg (two MRM transitions are shown).

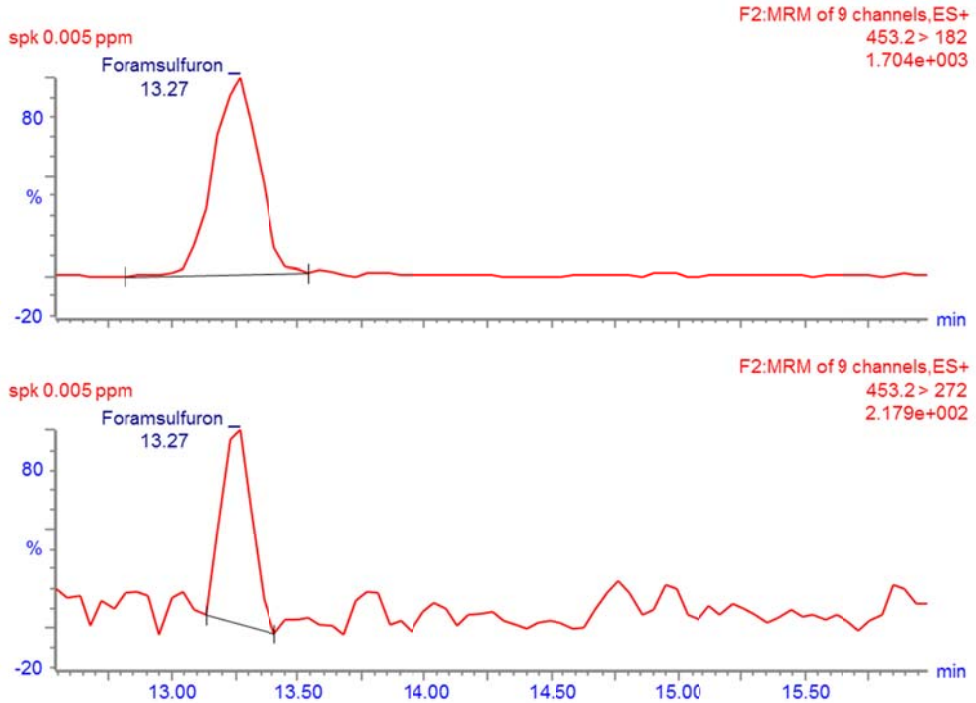

Figure 2: Examples of LC-MS/MS chromatograms foramsulfuron in maize for livestock feed obtained in positive mode when analysing extract spiked with 0.005 mg/kg (two MRM transitions are shown).

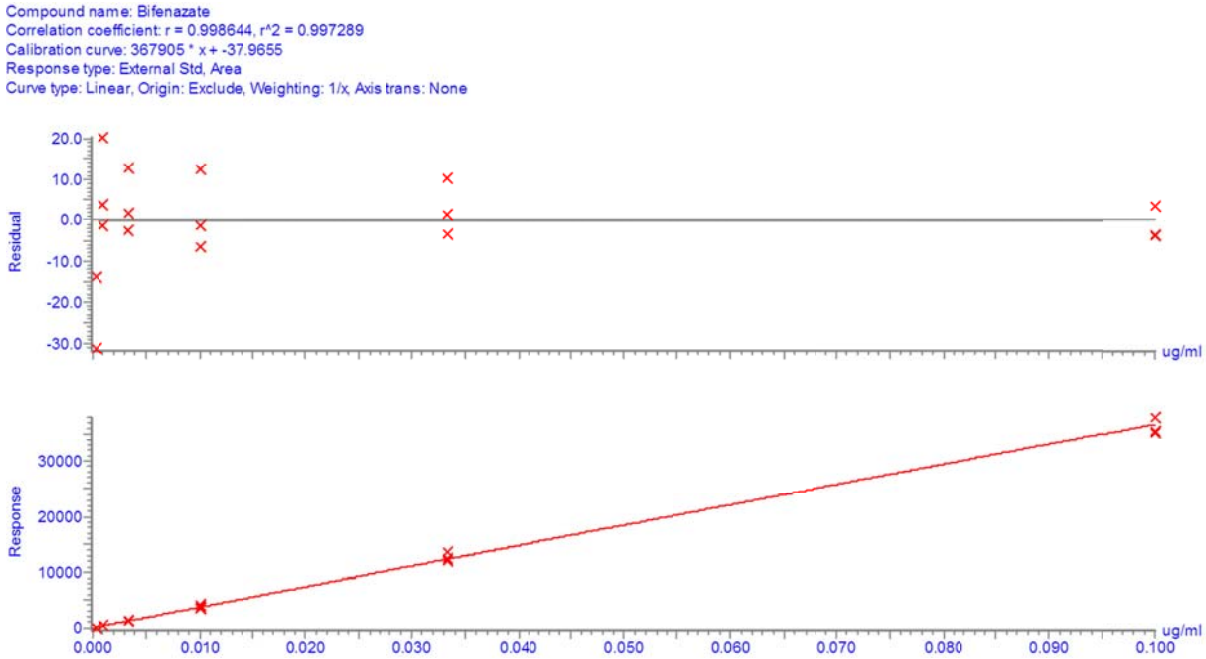

Figure 3. Examples of GC-MS/MS calibration curves for bifenazate matrix matched with maize for livestock feed (concentrations from 0.0003-0.1 µg/ml)

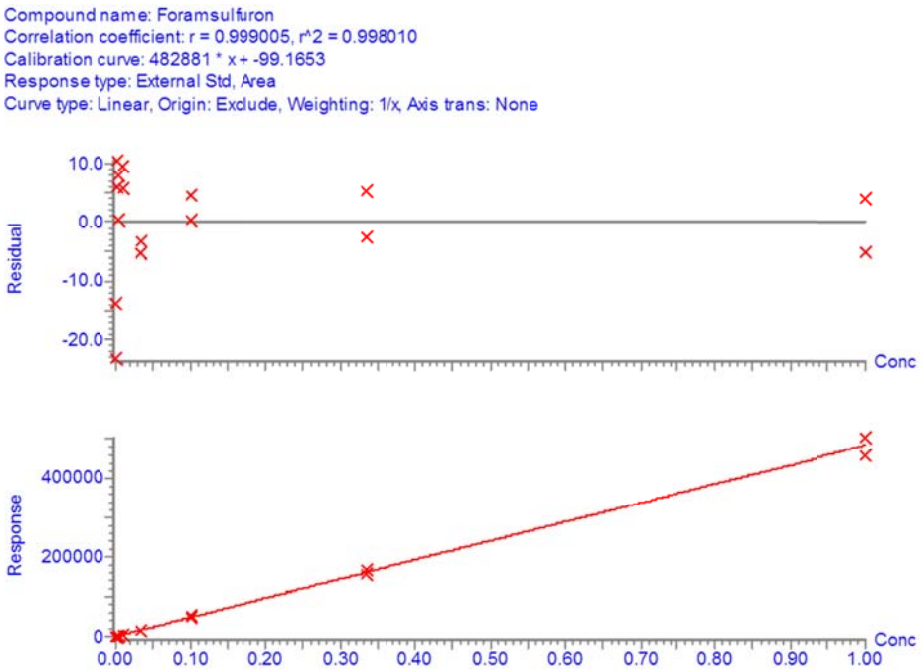

Figure 4. Examples of LC-MS/MS calibration curves for foramsulfuron matrix matched with maize for livestock feed (concentrations from 0.001-1.0 µg/ml).

5. Validation parameters

Precision – repeatability

Repeatability was calculated for all pesticides and degradation products on four spiking levels (0.005, 0.01, 0.02 and 0.1 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 (0.005, 0.01 mg/kg, 0.02 and 0.1 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 of the 11 compounds included in the validation study (Table 1) were successfully validated for maize on LC-MS/MS (8 analytes) or on both LC-MS/MS and GC-MS/MS (3 analytes), see **Appendix 2**.

For the accepted validation parameters the relative repeatability (RSD_r) varied between 3-18 % with an average on 9%. Recoveries was in the range of 52-111% at all concentration levels with an average on 86%. The LOQs were in the range of 0.005-0.1 mg/kg. Recoveries down to 50% was accepted if similar for all three spike levels and RSD_r were relatively low.

Bifenazate gave low recoveries on both GC-MS/MS and LC-MS/MS but acceptable RSD_r (5-12%) indicating low extraction recovery. Foramsulfuron also gave low recoveries (52-74%) but acceptable RSD_r (10-18%). Metabolites of spirotetramat (enol, glu, keto, mono) were possible to analyse by QuEChERS with LC-MS/MS detection, though spirotetramat-enol was only possible to validate at the highest spiking level (0.1 mg/kg) and still the recovery was relatively low (68%). Spirotetramat-glu was possible to validate at the second highest spiking levels (0.02 mg/kg).

8. Conclusions

In conclusion 11 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 generally 0.005 mg/kg though 0.01, 0.1 and 0.02 mg/kg for spiromesifen (LC-MS/MS), spirotetramat-enol and spirotetramat-glu, respectively.

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
Bifenazate	21.67	300	258	10	300	196	15
Fluopyram	15.5	173	145	5	396	223	10
Spiromesifen	21.3	272	254	10	272	209	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
Bifenazate	17.7	301	170	46	21	301	153	46	33
Fluopyram	17.70	397	173	45	25	397	145	45	45
Foramsulfuron	13.35	453	182	20	23	453	272	20	20
Iodosulfuron-methyl	13.81	530	163	21	13	530	390	21	14
Isoxadifen-ethyl	19.35	296	263	61	17	296	232	56	25
Spiromesifen	23.35	371	273	25	15	371	255	25	20
Spirotetramat	17.42	374	302	36	16	374	330	36	18
Spirotetramat-enol	13.3	302	216	46	28	302	270	46	24
Spirotetramat-glu	8.4	464	302	22	12	464	216	22	28
Spirotetramat-keto	14.64	318	300	24	16	318	268	24	20
Spirotetramat-mono	11.35	304	254	36	20	304	131	36	28

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

Numbers in italic is outside 70-120% recovery or above 20% RSD

		Spike level mg/kg 0.005			Spike level mg/kg 0.01			Spike level mg/kg 0.02			Spike level mg/kg 0.1		
		Recovery, %	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**	**LOQ**
GC	Bifenazate	79	12		70	8		*58*	5		*57*	6	0.005
LC	Bifenazate	*69*	5		*58*	12		*59*	9		*52*	7	0.005
GC	Fluopyram	111	5		109	5		97	6		101	3	0.005
LC	Fluopyram	107	8		102	6		103	9		97	5	0.005
LC	Foramsulfuron	74	13		*58*	10		*55*	17		*52*	18	0.005
LC	Iodosulfuron-methyl	100	11		89	4		85	9		82	3	0.005
LC	Isoxadifen-ethyl	88	17		98	5		99	9		98	5	0.005
GC	Spiromesifen	110	12		111	14		93	10		90	9	0.005
LC	Spiromesifen	79	33		98	15		94	10		93	14	0.01
LC	Spirotetramat	97	7		89	5		89	7		92	6	0.005
LC	Spirotetramat-enol	g									68	14	0.1
LC	Spirotetramat-glu	50	34		56	27		73	18		61	8	0.02
LC	Spirotetramat-keto	87	13		90	4		95	10		91	5	0.005
LC	Spirotetramat-mono	101	12		95	13		104	8		93	5	0.005

g) To low sensitivity

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.