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Validation Report 20

**Determination of pesticide residues in rice baby food
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 for 28 pesticides and metabolites in rice-based baby food. The pesticides included were selected mainly among pesticides with MRLs below 0.01 mg/kg or pesticides that are considered to be toxicological concern. Maximum Residue Limits, MRLs, have been implemented with Directive 2003/13/EC[1]. MRLs are applied to baby foods as proposed ready for consumption or as reconstituted according to the instructions from the manufacturers, thus no processing factors are needed. The aim of the validation was to achieve LOQs at MRL (see Table 1) or below. The QuEChERS extraction method has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit, vegetables and cereals[2].

2. Principle of analysis

Sample preparation: The rice based baby food was prepared according to the directions given on the package were 'ready for consumption' rice-based baby food.

Extraction: The sample was shaken with ACN and a salt and buffer mixture was added and the sample was shaken again.

Clean-up: After centrifugation the supernatant was transferred to a clean tube and put in -80 degree freezer. When the extract was almost thawed it was centrifuged and the supernatant was transferred to a tube containing PSA and $MgSO_4$. After shaking and an additional centrifugation step the "final extract" was diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the matrix matched calibration standards. These extracts were then analysed by LC-MS/MS.

In order to obtain detectable peaks for the GC-MS/MS amenable compounds and in attempt to increase the sensitivity for the LC-MS/MS amenable compounds a modified QuEChERS were also employed. This modified method involved a concentration step. A five ml aliquot of the "final extract" was evaporated using a gentle flow of nitrogen to almost dryness and reconstituted in 1 ml acetonitrile and were not diluted 1:1 with acetonitrile, thus concentrated 10 times compared to the above described procedure. The matrix blank was concentrated 5 times and then diluted 1:1 with the standard calibration solutions in order to obtain the same matrix concentration in the calibration solutions as in the samples.

Quantification and qualification: The final concentrated extracts were analysed by GC/MS/MS and LC-MS/MS. The non-concentrated extracts were only analysed by LC-MS/MS.

GC-MS/MS: The GC–MS/MS analysis was performed on a Quattro Micro Tandem GC–MS/MS (Waters, USA). The system consisted of a PAL-GC Auto sampler, an Agilent GC 6890N and a

Quattro Micro Tandem mass spectrometer. The pesticide residues were 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. 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 LC-MS/MS analysis was performed on a HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) connected to a Micromass Quattro Ultima Triple Quadrupole Instrument. The analytes were separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI). The present validation only include pesticides determined in positive mode. All pesticides were detected in the MRM mode. For each pesticide or metabolite a 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 sought validated for 28 pesticides or metabolites in rice-based baby food aiming at LOQs at or below the relevant MRLs (see **Table 1).** Depending on the toxicity of the pesticides, different MRLs were assigned to each pesticides by Directive 2003/13/EC. The validation was performed on 5-6 replicates on rice-based baby food at each of the three spiking levels; 0.001, 0.005 and 0.01 mg/kg. A blank sample of rice-based baby food was included.

Table 1. Pesticides included in the recovery experiments.

	Pesticides compounds	MRL according to Directive 2003/13/EC
1	3-Hydroxy carbofuran	0.01
2	Aldrin	Shall not be used
3	Cadusafos	0.006
4	Carbofuran	0.01
5	Demeton-S-methyl	0.006
6	Oxydemeton-methyl (Dementon-S-methyl sulfoxide)	0.006
7	Demeton-S-methyl sulfone	0.006
8	Diazinon	0.01
9	Dichlorvos	0.01
10	Dieldrin	Shall not be used
11	Dimethoate	0.01
12	Disulfoton	Shall not be used
13	Disulfoton sulfone	Shall not be used
14	Disulfoton sulfoxide	Shall not be used
15	Endrin	Shall not be used
16	Ethoprophos	0.008
17	Fensulfothion	Shall not be used
18	Fipronil	0.004
19	Fipronil- desulfinyl	0.004
20	Haloxyfop	Shall not be used
21	Heptachlor	Shall not be used
22	Heptachlorepoxide-cis	Shall not be used
23	Heptachlorepoxide-trans	Shall not be used
24	Hexachlorobenzene	Shall not be used
25	Nitrofen	Shall not be used
26	Omethoate	Shall not be used
27	Terbufos	Shall not be used
28	Triazoxide	0.01

4. Chromatograms and calibration curves

The calibration curve was prepared by the analysis of each of the analytes at least 4 calibration levels, i.e. 0.001, 0.003, 0.01 and 0.03 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 obtained when analysing the extracts by GC-MS/MS are presented in **Figure 1-4**. Examples of calibration curves for LC-MS/MS are presented in **Figure 5-8**.

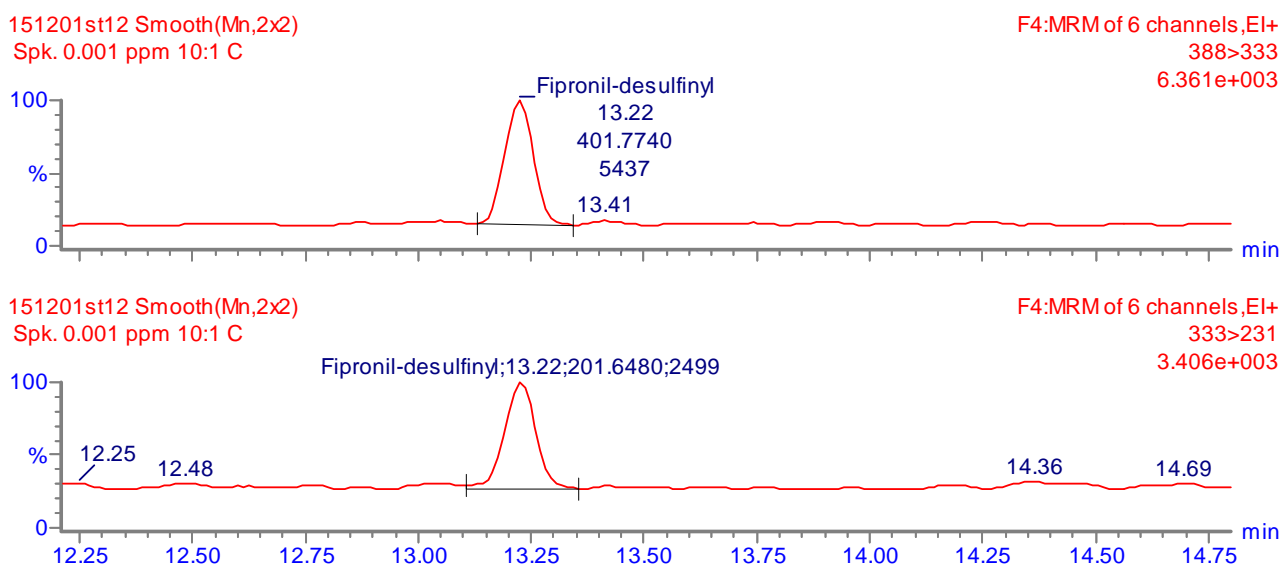**Figure 1:** Examples of GC-MS/MS chromatograms for fipronil-desulfinyl in in rice-based baby food obtained when analysing extract spiked with 0.001 mg/kg (up-concentrated at 10:1). Both MRM transitions are shown.
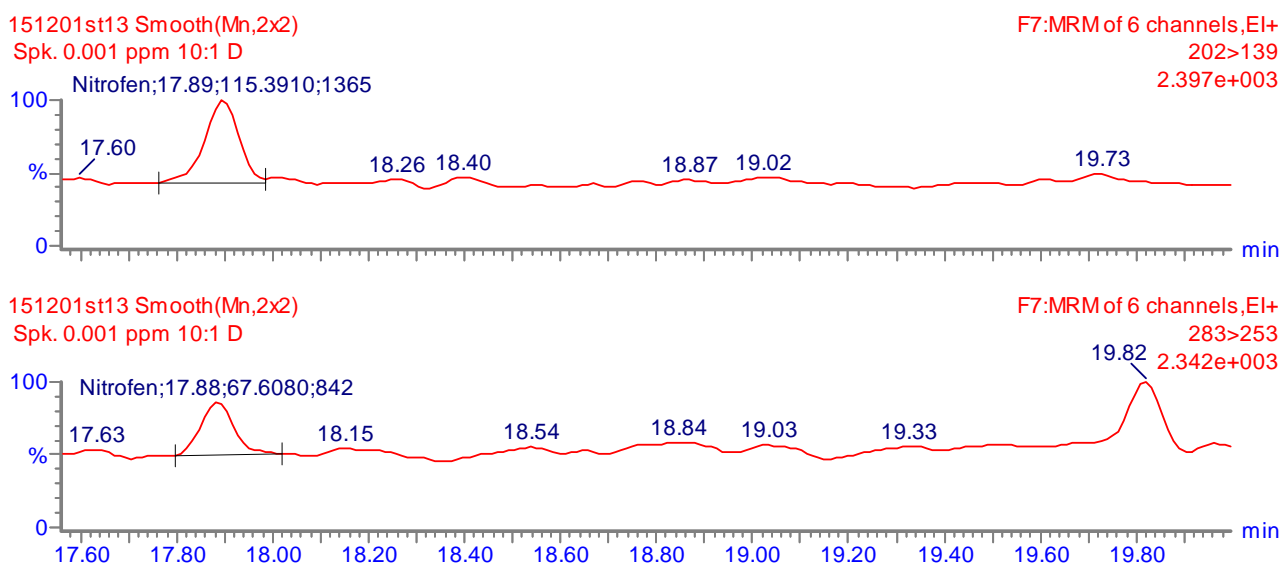

Figure 2: Examples of GC-MS/MS chromatograms nitrofen in rice-based baby food obtained when analysing extract spiked with 0.001 mg/kg (up-concentrated at 10:1). Both MRM transitions are shown.

Figure 3: Examples of LC-MS/MS chromatograms demeton-S-methyl sulfone in rice-based baby food obtained in positive mode when analysing extract spiked with 0.001 mg/kg (up-concentrated at 10:1). Both MRM transitions are shown for each pesticide.

Figure 4: Examples of LC-MS/MS chromatograms carbofuran in rice-based baby food obtained when analysing extract in positive mode spiked with 0.001 mg/kg (up-concentrated at 10:1). Both MRM transitions are shown.
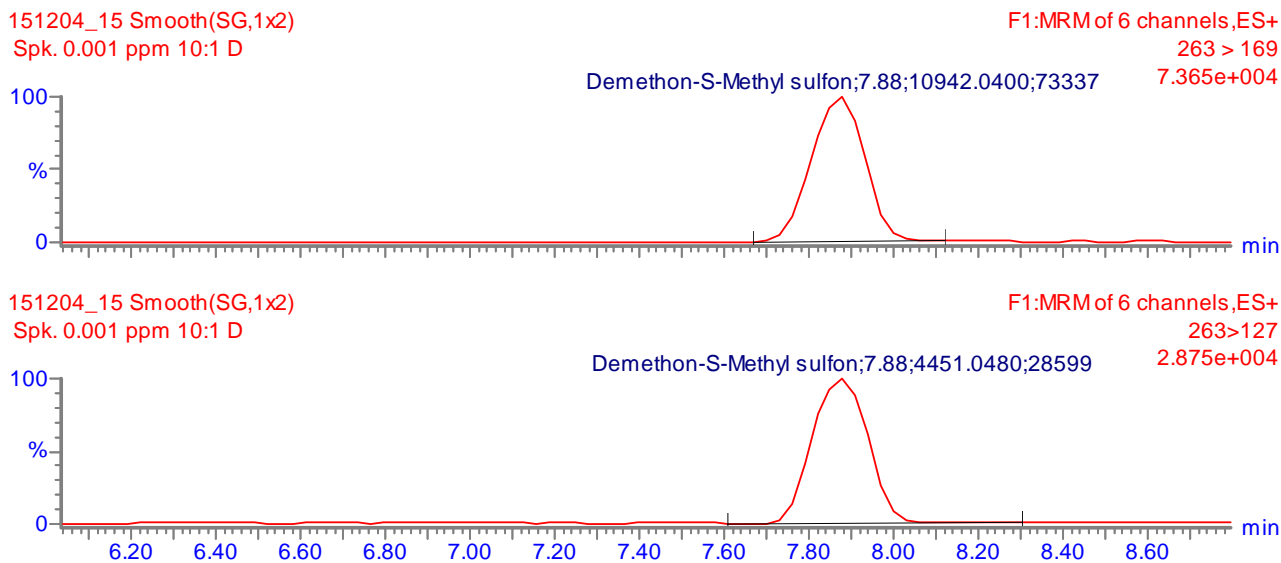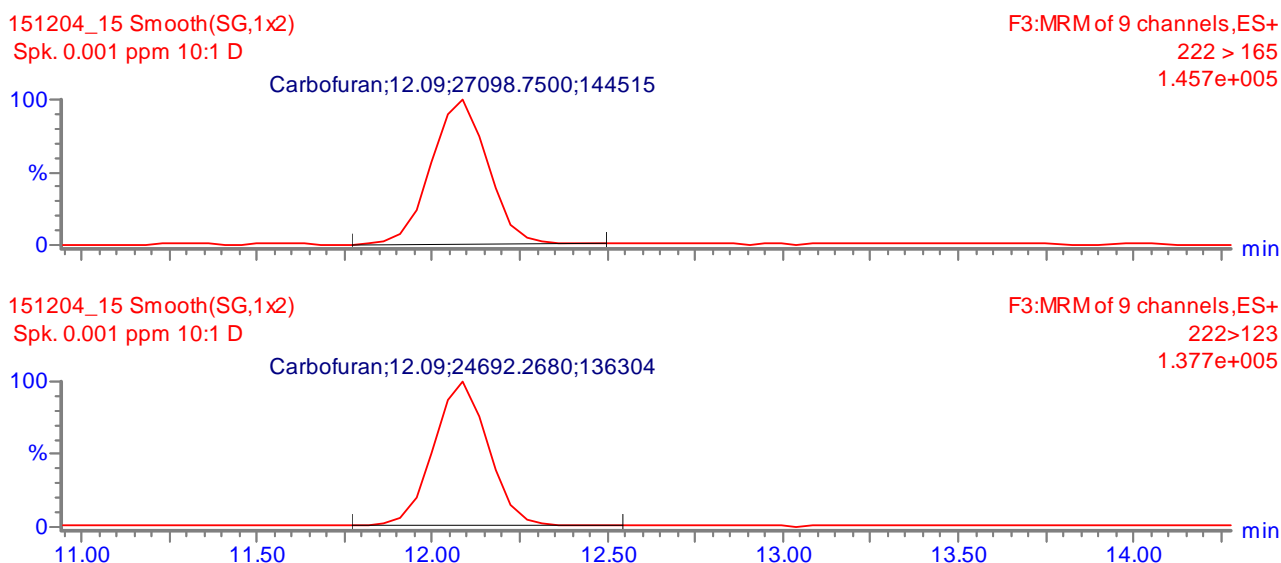
Compound name: Fipronil-desulfinyl
Coefficient of Determination: R^2 = 0.999491
Calibration curve: -2.01028e+006 * x^2 + 624957 * x + -86.3395
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 5. Examples of GC-MS/MS calibration curves for fipronil-desulfinyl matrix matched with rice-based baby food (concentrations from 0.001-0.03 µg/ml).

Compound name: Nitrofen
Correlation coefficient: r = 0.997757, r^2 = 0.995519
Calibration curve: 170631 * x + 10.7327
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 6. Examples of GC-MS/MS calibration curves for nitrofen matrix matched with rice-based baby food (concentrations from 0.001-0.03 µg/ml).

Compound name: Demethon-S-Methyl sulfon
Correlation coefficient: r = 0.998295, r^2 = 0.996594
Calibration curve: 3.67712e+006 * x + 137.492
Response type: External Std, Area
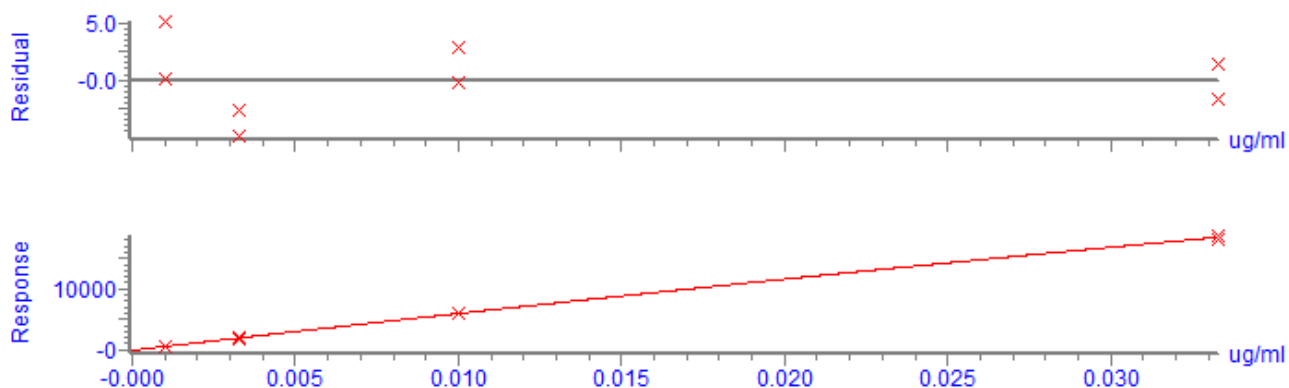Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
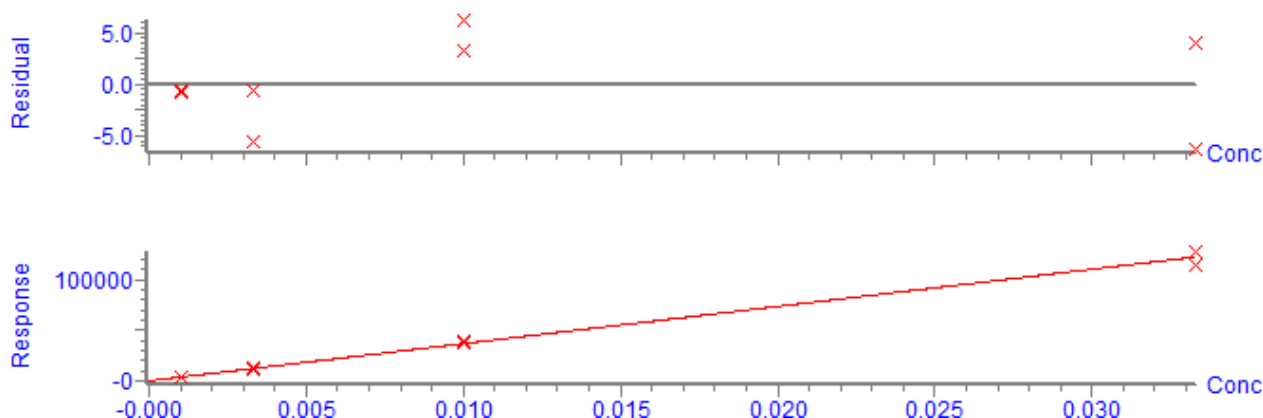
Figure 7. Examples of LC-MS/MS calibration curves for demeton-S-methyl sulfone matrix matched with rice-based baby food (concentrations from 0.001-0.03 µg/ml).

Compound name: Carbofuran
Correlation coefficient: r = 0.999362, r^2 = 0.998725
Calibration curve: 8.37112e+006 * x + 165.061
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

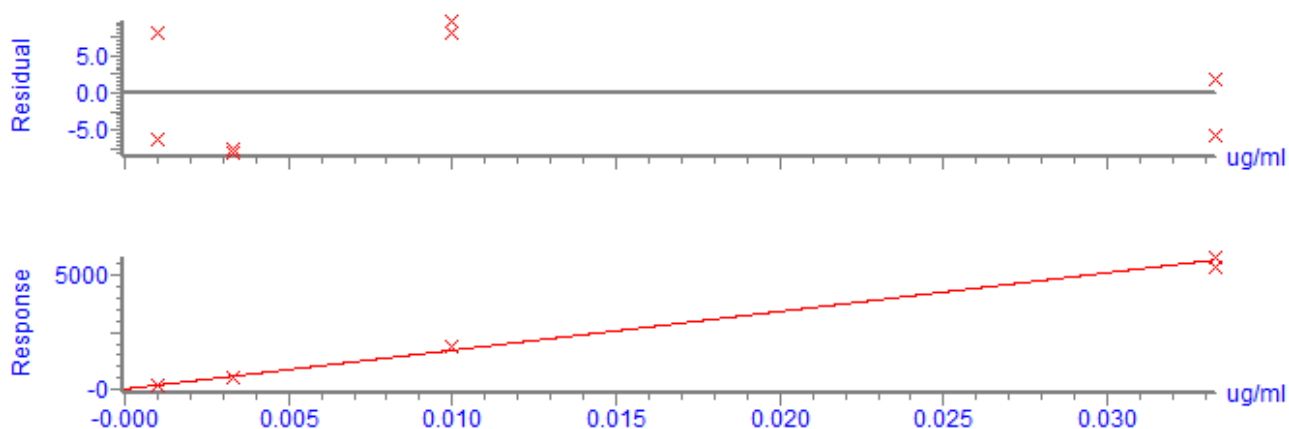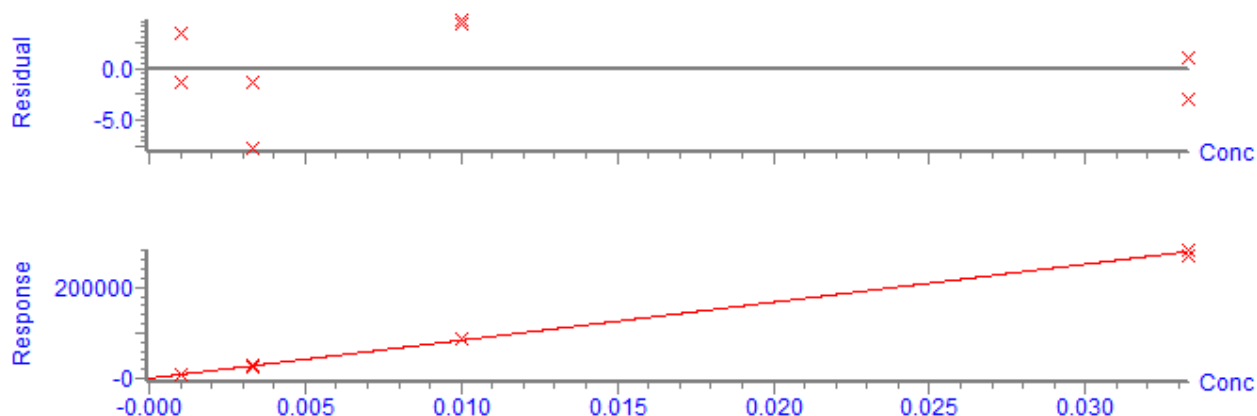**Figure 8.** Examples of LC-MS/MS calibration curves for carbofuran matrix match with rice-based baby food (concentrations from 0.001-0.03 µg/ml).

5. Validation parameters

Accuracy – Recovery
The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.001 mg/kg, 0.005 mg/kg and 0.01 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 and limit of quantification (LOQ) using non-concentrated and concentrated extracts are presented in appendix 2 and 3, accordingly.

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 ≤20%[3].
2. The average relative recovery must be between 70 and 120%[3].

7. Results and discussion

Validation on rice-based baby food

Results obtained using LC-MS/MS

Of the 28 compounds included in the validation study (Table 1) were 15 compounds successfully validated on rice-based baby food analysed by LC-MS/MS using the standard QuEChERS procedure outlined in Appendix 4. The validation results are presented in **Appendix 2.**

The LOQs were 0.001 for most compounds, though for demethon-S-methyl and triazoxide a LOQ of 0.005 mg/kg was obtained and for 3-hydroxy carbofuran and dichlorvos an LOQ of 0.01 was obtained. Disulfoton and haloxyfop were not possible to validate using the standard QuEChERS and analysis by LC-MS/MS due to low recoveries (<50%). Recoveries of all the other compounds were in the range of 70-130% for all three spike levels. For 15 of the 28 compounds the obtained LOQs were lower or equal to the assigned MRLs.

Results obtained by including concentration step and analysis by GC-MS/MS

Of the 28 compounds included in the validation study (Table 1) were 13 compounds successfully validated on rice-based baby food analysed by GC-MS/MS by including concentration step in the standard QuEChERS procedure, see **Appendix 3**.

The LOQs were 0.001 for most compounds, though for diazinon, dieldrin, disulfoton, endrin, fipronil and terbufos a LOQ of 0.005 mg/kg was obtained and for carbofuran, dichlorvos and heptachlor epoxide-trans (GC) an LOQ of 0.01 was obtained. Recoveries of cadusafos, disulfoton, fipronil-desulfinyl, nitrofen, and terbufos were in the range of 70-120% for all three spike level. Though for other compounds the results should consequently, be corrected for recoveries.

Six of the compounds included in the study were not possible to validate using GC-MS/MS: aldrin carbofuran, dimethoate, disulfoton sulfone, fensulfothion and hexachlorobenzene (HCB) due to low sensitivity. For nine of the 28 compounds the obtained LOQs were higher than the assigned MRLs. The GC-MS/MS used is 10 year old. Thus if a newer and therefore also more sensitive instrument was available it is likely that more compounds could be validated and/or lower LOQ could be obtained.

Results obtained by including concentration step and analysis by LC-MS/MS

Of the 28 compounds included in the validation study (Table 1) were 15 compounds successfully validated on rice-based baby food using the modified QuEChERS method and LC-MS/MS, see **Appendix 3**.

The LOQs were 0.001 for most compounds, though for 3-hydroxy carbofuran, carbofuran, demethon-S-methyl sulfone, diazinon, dichlorvos, dimethoate, disulfoton sulfone, disulfoton sulfoxide, ethoprophos and oxydemethon methyl a LOQ of 0.005 mg/kg was obtained. Recoveries were in the range of 70-120% for all three spike levels, except for carbofuran, demethon-S-Methyl sulfon, dichlorvos, dimethoate, disulfoton sulfoxide and oxydemethon methyl and heptachlor. Therefore, the results should be corrected for recoveries.

Two of the compounds (haloxyfop and disulfoton) included in the study were not possible to validate using LC-MS/MS. In general, lower LOQ of 0.001 was achieved for most of the compounds using LC-MS/MS. For five of the 28 compounds the obtained LOQs were higher than the assigned MRLs. The LC-MS/MS system employed is also approximately 10 years old but surprisingly low levels could be detected with this instrument.

8. Conclusions

In conclusion 25 pesticides and metabolites were successfully validated on rice-based baby food using the QuEChERS method and GC-MS/MS or/and LC-MS/MS. The LOQs obtained were 0.001 mg/kg except for 3-hydroxy carbofuran (LC), carbofuran (LC), demethon-S-methyl sulfone (LC), diazinon (LC and GC), dichlorvos (LC), dieldrin (GC), dimethoate (LC), disulfoton (GC), disulfoton sulfone (LC), disulfoton sulfoxide (LC), endrin (GC), ethoprophos (LC), fipronil (GC), oxydemethon methyl (LC) and terbufos (GC) a LOQ of 0.005 mg/kg was obtained and for carbofuran (GC), dichlorvos (GC), disulfoton sulfone (GC), and heptachlorepoxide-trans (GC) an LOQ of 0.01 was obtained. For nine (aldrin, dieldrin, disulfoton, endrin, fipronil, haloxyfop, heptachlor epoxide-trans, hexachlorobenzene, terbufos) of the 25 validated compounds the obtained LOQs were higher than the assigned MRL. Thus the presented method was found not to applicable for the control analysis of residues of these nine pesticides in baby food. In order to obtain acceptable validation results for most of the GC amenable pesticides a concentration step was needed.

Better recoveries and lower RSDr were achieved at spike level of 0.005 mg/kg for almost all pesticides than at spike levels of 0.001 mg/kg. This study showed that lower LOQ is achieved by up-concentration of extract at 10:1 using the modified QuEChERS procedure. Especially for the GC-MS/MS amendable compounds better results were obtained due to improved method. Though, inclusion of the concentration step also introduced higher RSDr.

In general, lower LOQ of 0.001 was achieved for most of the compounds using LC-MS/MS. Fifteen out of 25 validated compounds were LC amendable compounds. For dimethoate, disulfoton sulfone and fensulfothion, lower LOQs could be obtained when analyzing by LC-MS/MS than when analyzing by GC-MS/MS.

9. References

1 Directive 2003/13/EC: Commission Directive 2003/13/EC of 10 February 2003 amending Directive 96/5/EC on processed cereal-based foods and baby foods for infants and young children.

2 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

3 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/12571/2013, 01/01/2014, 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
Aldrin	14.08	293	258	20	293	223	20
Cadusafos	10.30	159	131	5	158	114	5
Carbofuran	7.5	149	121	5	164	149	10
Diazinon	11.6	304	179	10	276	179	10
Dichlorvos	7.1	109	79	5	187	93	10
Dieldrin	17.16	380	345	7	279	243	15
Hexachlorobenzene	10.57	284	248	15	286	250	15
Dimethoate	10.9	229	87	7	125	79	6
Disulfoton	11.77	274	245	10	186	153	5
Disulfoton sulfone	16.21	213	153	5	213	125	10
Endrin	17.85	281	245	5	263	227	15
Fensulfothion	18.39	308	97	20	293	125	15
Fipronil	15.6	367	213	20	367	255	15
Fipronil-desulfinyl	13.23	388	333	20	333	231	30
Heptachlor	12.8	272	237	10	274	238	10
Heptachlor epoxide-cis	14.88	353	263	15	351	261	15
Heptachlor epoxide-trans	15.00	353	252	15	351	251	20
Nitrofen	17.89	202	139	21	283	253	15
Terbufos	11.35	288	231	15	231	203	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
3-Hydroxy carbofuran	9.27	255.3	163	30	13	255.3	107	30	25
Cadusafos	20.93	271.3	159	50	13	271.3	97	50	40
Carbofuran	12.12	222	165	10	10	222	123.0	20	20
Demeton-S-methyl	12.39	231	89	21	5	231	61.3	20	25
Demeton-S-methyl sulfon	8.06	263	169	55	15	263	127	45	28
Oxydemeton methyl (Dementon-S-methyl sulfoxide)	7.71	247	169	33	10	247	127	18	25
Diazinon	19.89	305	169	20	20	305	153	20	20
Dichlorvos	12.05	221	108.9	20	20	221	127	20	20
Dimethoate	9.5	230	125	20	20	230	170.9	20	20
Disulfoton	21.1	275.1	89	20	20	275.1	61	20	20
Disulfoton sulfone	14.21	307.4	152.8	25	10	307.4	124.8	20	20
Disulfoton sulfoxide	13.81	291	184.8	25	15	291	212.8	25	10
Ethoprophos	18.24	243	97	50	23	243	131	50	31
Fensulfothion	14.66	309.1	173	20	20	309.1	252.9	20	20
Haloxyfop	17.94	379	316	22	21	379	91.1	22	39
Omethoat	7.26	214	183	10	11	214	143	10	17
Triazoxide	15.14	248	124	50	30	248	150	50	30

Appendix 2. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQ) for pesticides validated on rice-based baby food using QuEChERS.
Numbers in italic is outside 70-120% recovery or above 20% RSD

	QuEChERS	Spike level, mg/kg 0.001		Spike level, mg/kg 0.005		Spike level, mg/kg 0.01			**MRL according to Directive 2003/13/EC**
		Recovery %	**RSDr %**	**Recovery %**	**RSDr %**	**Recovery %**	**RSDr %**	**LOQ**	
LC	3-Hydroxy Carbofuran	*73*	*135*	*72*	*42*	90	7	0.01	0.01
LC	Cadusafos	107	19	101	4	119	17	0.001	0.006
LC	Carbofuran	107	19	108	4	120	18	0.001	0.01
LC	Demethon-S-Methyl	*120*	*42*	89	17	130	14	0.005	0.006
LC	Demethon-S-Methyl sulfone	107	19	104	0	110	15	0.001	0.006
LC	Diazinon	107	19	97	3	118	17	0.001	0.01
LC	Dichlorvos	*27*	*245*	*43*	*74*	95	30	0.01	0.01
LC	Dimethoate	113	14	104	0	113	15	0.001	0.01
LC	Disulfoton sulfone	120	21	116	6	107	19	0.001	Shall not be used
LC	Disulfoton sulfoxide	113	14	107	4	120	18	0.001	Shall not be used
LC	Ethoprophos	100	22	104	5	120	14	0.001	0.008
LC	Fensulfothion	120	21	125	3	121	17	0.001	Shall not be used
LC	Omethoat	100	22	87	7	90	12	0.001	Shall not be used
LC	Oxydemethon methyl	107	19	93	4	102	16	0.001	0.006
LC	Triazoxide	*20*	*167*	87	12	108	20	0.005	0.01
Pesticides/metabolites not possible to validate									
LC	Disulfoton	a, d							
LC	Haloxyfop	a, d							

a) RSDr > 20%; b) Not GC-MS/MS amenable; c) Not LC-MS/MS amenable; d) Recovery <50%; e) Recovery >50%; f) Not multimethod amenable; g) Too low sensitivity; h) Not multimethod amenable ; i) Interfering matrix enables quantification; j) LOQ> assigned MRL; *LOQs > MRLs

Appendix 3. Appendix 2. Recoveries, repeatability (RSDr) and Limit of Quantification (LOQ) for pesticides validated on rice-based baby food using modified QuEChERS (concentrated at 10:1).

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

	QuEChERS	Spike level, mg/kg 0.001		Spike level, mg/kg 0.005		Spike level, mg/kg 0.01		LOQ	MRL according to Directive 2003/13/EC
		Recovery %	RSDr %	Recovery %	RSDr %	Recovery %	RSDr %		
LC	3-hydroxy carbofuran	126	17	96	16	80	14	0.005	0.01
GC	Aldrin*	*2*	*224*	*36*	*19*	*47*	*18*	-	Shall not be used
LC	Cadusafos	120	24	101	13	91	13	0.001	0.006
GC	Cadusafos	113	23	93	13	84	19	0.001	0.006
LC	Carbofuran	*168*	*53*	106	11	94	13	0.005	0.01
GC	Carbofuran	*175*	*60*	*142*	*51*	*109*	*28*	-	0.01
LC	Demethon-S-methyl	106	22	82	12	68	18	0.001	0.006
LC	Demethon-S-methyl sulfone	*127*	*27*	99	10	88	13	0.005	0.006
LC	Diazinon	*121*	*31*	98	14	90	12	0.005	0.01
GC	Diazinon	*126*	*26*	105	14	100	32	0.005	0.01
LC	Dichlorvos	*111*	*61*	94	16	99	13	0.005	0.01
GC	Dichlorvos	*177*	*32*	*105*	*27*	93	14	0.01	0.01
GC	Dieldrin*	*170*	*55*	82	17	135	16	0.005	Shall not be used
LC	Dimethoate	*138*	*34*	101	11	89	12	0.005	0.01
GC	Dimethoate*	*290*	*132*	*21*	*172*	*7*	*245*	-	0.01
LC	Disulfoton*	*108*	*144*	*45*	*131*	*60*	*39*	-	Shall not be used
GC	Disulfoton*	*107*	*38*	94	18	109	33	0.005	Shall not be used
LC	Disulfoton sulfone*	*125*	*20*	108	12	97	15	0.005	Shall not be used
GC	Disulfoton sulfone*	*86*	*24*	*165*	*4*	*111*	*39*	-	Shall not be used
LC	Disulfoton sulfoxide*	*149*	*37*	105	14	92	13	0.005	Shall not be used
GC	Endrin*	*116*	*98*	75	10	75	22	0.005	Shall not be used
LC	Ethoprophos	*122*	*27*	102	13	93	12	0.005	0.008
LC	Fensulfothion*	111	22	106	12	95	14	0.001	Shall not be used

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	QuEChERS	Spike level, mg/kg 0.001		Spike level, mg/kg 0.005		Spike level, mg/kg 0.01			**MRL according to Directive 2003/13/EC**
		Recovery %	RSDr %	Recovery %	RSDr %	Recovery %	RSDr %	LOQ	
GC	Fensulfothion*	*26*	*36*	*29*	*24*	*21*	*33*	-	Shall not be used
GC	Fipronil*	*158*	*27*	116	13	79	14	0.005	0.004
GC	Fipronil-desulfinyl	118	19	118	12	91	17	0.001	0.004
LC	Haloxyfop*	*51*	*32*	*45*	*8*	*45*	*22*	-	Shall not be used
GC	Heptachlor	60	21	61	13	55	23	0.001	Shall not be used
GC	Heptachlorepoxide cis	85	22	82	12	64	14	0.001	Shall not be used
GC	Heptachlorepoxide trans*	*119*	*52*	*89*	*30*	62	15	0.01	Shall not be used
GC	Hexachlorobenzene (HCB)*	*50*	*26*	*42*	*10*	*36*	*25*	-	Shall not be used
GC	Nitrofen	90	19	89	11	64	15	0.001	Shall not be used
LC	Omethoat	102	25	85	9	74	16	0.001	Shall not be used
LC	Oxydemethon Methyl*	*126*	*31*	92	15	81	8	0.005	Shall not be used
GC	Terbufos*	*91*	*37*	89	14	94	26	0.005	Shall not be used
LC	Triazoxide	103	21	94	14	85	14	0.001	0.01
Pesticides/metabolites not possible to validate									
LC	Haloxyfop	a, d							
GC	Aldrin	a, d, g							
GC	Hexachlorobenzene (HCB)	d, g							

a) RSDr > 20%; b) Not GC-MS/MS amenable; c) Not LC-MS/MS amenable; d) Recovery <50%; e) Recovery >50%; f) not multimethod amenable; g) To low sensitivity; h) not multimethod amenable ; i) interfering matrix enables quantification; j) LOQ> assigned MRL, *LOQs > MRLs.

Appendix 4: Principles of the QuEChERS method for cereal 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.