CRL for Cereals and Feeding stuff
National Food Institute
Danish Technical University

Method validation report 2

Determination of pesticides in cereals using the QuEChERS method and GC-ITD

Second version - includes correction of some of the LOQs

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Contents

1. Introduction

This report describes the validation of the QuEChERS method combined with GC-ITD for determination of pesticide residues in cereals.

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 has already been validated on fruits and vegetables[1], but the data available on cereals is limited.

The method validated here is based on the procedure for dry matrixes (<30% water content) according to the document CEN/TC 275/WG 4 N 0204 (CEN document)(available as a draft). Even though cereals have a fat content of about 2%[2] no attempt has been made to remove the fat from the extract, e.g. freezing out as proposed in the CEN document, since no problems caused by fat has been observed.

2. Principle of analysis

Cold water/ice water, acetonitril and an internal standard are added to the milled sample. The sample is shaken and a salt and buffer mixture is added and the sample is shaken again. After centrifugation the supernatant is transferred to a tube with PSA and $MgSO_4$. After shaking and an additional centrifugation step the extract is analysed by GC-ITD and large volume injection. The injection volume was 8 µl. Instrument specifications as setting are presented in details in Poulsen and Granby 2000[3].

3. Validation design

The method was validated for 83 pesticides, isomers or degradation products in four types of flour, oat, rice, rye and wheat.

The validation was performed at three concentration levels as double determinations. The concentration levels were 0.01, 0.02 and 0.2 mg/kg. Thus a total of 6 samples per flour type were spiked and analyzed. A blank sample was included for each matrix. The experiments were carried out once on oat, rice and rye and twice on wheat, in total 5 experiments (See Table 1). The experiments were performed by two different technicians and on different days.

Table 1 Validation design, spike levels and matrices

Experiment	0 mg/kg	0.01 mg/kg		0.02 mg/kg		0.2 mg/kg	
1 – wheat	x	x	x	x	x	x	x
2- rye	x	x	x	x	x	x	x
3- rice	x	x	x	x	x	x	x
4 -maize	x	x	x	x	x	x	x
5- wheat	x	x	x	x	x	x	x

4. Calibration curves

The calibration curve is determined by the analysis of each of the 83 pesticides at 5 calibration levels, i.e. 0.00289, 0.0087, 0.0289, 0.0868 and 0.289 µg/ml. The calibration curves were best fitted to a linear curve. The majority of the correlation coefficients (R) were higher or equal to 0.98. Examples of calibration curves are presented in Figure 1.

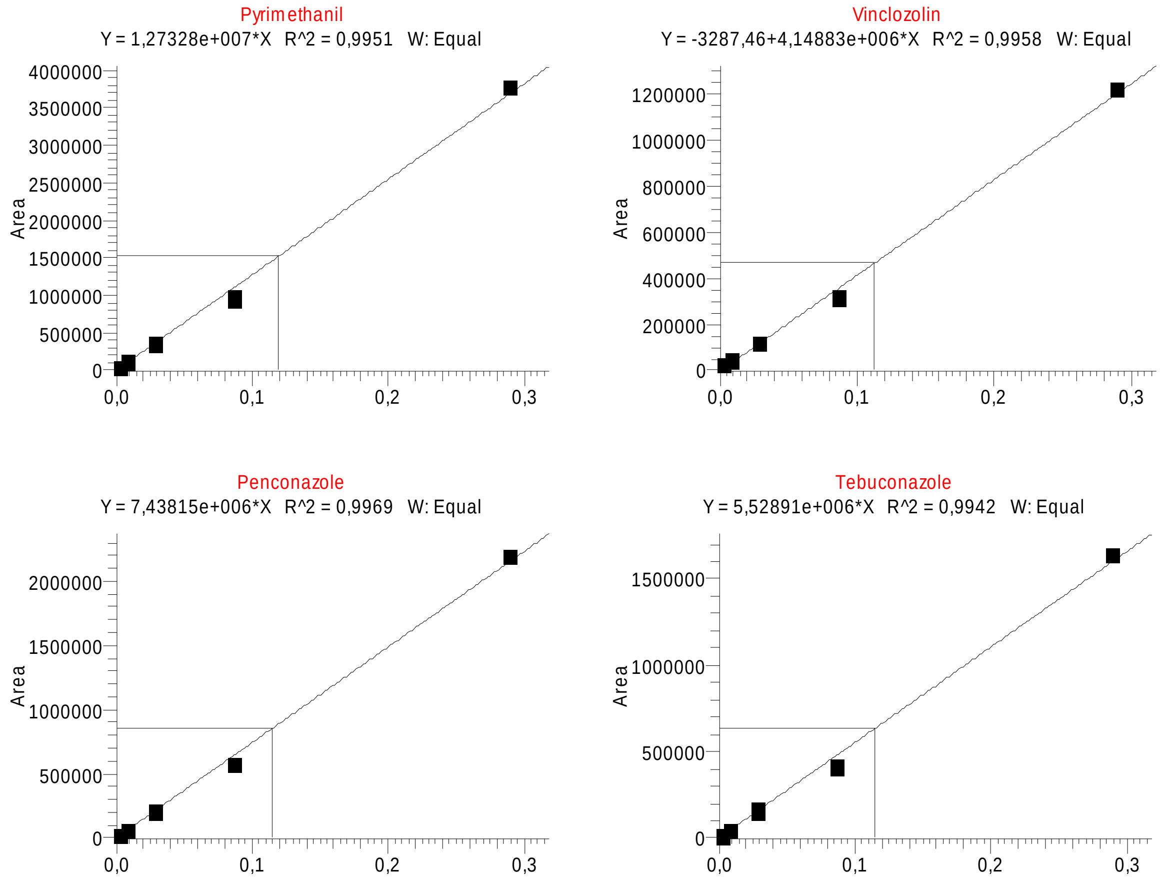

Figure 1: Calibration curves for pyrimethanil, vinclozolin, penconazole and tebuconazole.

5. Precision - repeatability and reproducibility

As precision often varies with analyte concentration, repeatability and in-house reproducibility were calculated for all matrices and all pesticides and degradations products at all three spiking levels.

The repeatability is given as the relative standard deviation on the results from two or more analysis of identical samples, by the same operator, on the same instrument and within a short period of time. Repeatability is calculated from the double determinations.

In-house reproducibility is relative standard deviation on results obtained under reproducibility conditions, with the same method on the same sample by different operators within a larger period of time. The In-house reproducibility is a combination of the repeatability variance and the in-house reproducibility.

In appendix 1 are the calculated values for repeatability and In-house reproducibility presented for the validated compounds.

The repeatability and reproducibility has been calculated in accordance to ISO 5725-2[4].

6. Accuracy - Recovery

Certified reference material is not available for all pesticides in all matrices. In the absence of reference materials, trueness has been calculated as the recovery of the validated compounds from the four cereal matrices at the three spiking levels.

The recoveries for each of the validated compounds are presented in Appendix 1.

7. 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.

8. 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 standard deviation of the relative repeatability and reproducibility must be less than or equal to the standard deviation proposed by Horwitz[5].
2. The average relative recovery must be between 70 and 110%[6].

If the above mentioned criteria have been met, the detection limits have been calculated.
An example of accepted results (repeatability, reproducibility and recovery) is shown in Table 2.

Table 2: Example of accepted results for repeatability, with-in laboratory reproducibility and Horwitz standard deviations

	Cereals		
Spiking level (mg/kg)	0.011	0.022	0.217
Number of results	10	10	10
Repetitions	5	5	5
Recovery (mg/kg)	0.012	0.024	0.218
Recovery (%)	108	109	100
S_r (mg/kg)	0.0005	0.0015	0.0065
RSD_r (%)	4.1	6.2	3.0
S_R (mg/kg)	0.0012	0.0030	0.0165
RSD_R (%)	9.9	12.4	7.2
$RSD_{Horwitz}$	31.5	28.4	20.1

Recovery (mg/kg): mean absolute recovery in mg/kg. Recovery (%): Mean relative recovery in %. S_r (mg/kg): The standard deviation on the absolute repeatability in mg/kg. RSD_r, (%): The standard deviation on the relative repeatability in mg/kg. S_R (mg/kg): The standard deviation on the absolute reproducibility in mg/kg. RSD_R (%): The standard deviation on the relative reproducibility in mg/kg. $RSD_{Horwitz}$: the Horwitz value at the relevant concentration.

9. Limit of quantification, LOQ

The calculation of the limit of quantification (LOQ) has been based on the results of the lowest spiking level for which the results met the acceptance criteria, as six times the standard deviation of the absolute recoveries.

The limits of quantification for the pesticides included in the validation are presented in Appendix 1. The ions used for quantification are presented in Appendix 2.

10. Results

The QuEChERS method, in accordance to CEN/TC 275/WG 4 N 0204, has been tested for 83 pesticides, isomers and degradation products in cereal flour, represented by oat, rice, rye and wheat.

The criteria for acceptance were met for 62 out of 83 pesticides, isomers and degradation products. The LOQs ranged from 0.006 mg/kg to 0.24 mg/kg with a median at 0.014 mg/kg. Some of the compounds could only be validated at the highest fortification level (0.217 mg/kg) or at the second highest fortification levels (0.022 mg/kg), and in several cases this was due to high recovery at the lower levels.

The criteria for acceptance were not met for 21 of the compounds. Results for binapacryl, fenamiphos, fludioxonil, flutolanil, hexaconazole and iodofenphos did not meet the acceptance criteria due to interfering matrix peaks in all four types of flour. Besides these six pesticides it was not possible to quantify diethofencarb, flusilazole and kresoxim-methyl in rice samples because of interfering matrix peaks. A large matrix peak was observed in rice samples at a retention time of about 14 minutes to about 16 minutes indicating the clean up was not sufficient for rice. A chromatogram of a spiked rice sample is shown in Appendix 3.
Another fifteen compounds did not elute in one of the large matrix peaks, but still could not meet the acceptance criteria. For some of these compounds the ion ratios were low compared to the noise ratio resulting in high repeatability and reproducibility. For other compounds the repeatability was acceptable whereas the reproducibility was considerately higher than the relevant Horwitz value.

The results for the different pesticides which were accepted are listed in Appendix 1.

It is expected that the problems with interfering matrix could partly be eliminated if the extracts were analysed on a MS quadropol instrument. Further analysis will be performed to eliminate the problems and meet the acceptance criteria for the remaining 21 pesticides.

11. Conclusion

The method is validated for 62 pesticides, isomers or degradation products. The limits of quantificaiton ranged from 0.006-0.24 mg/kg, with a median at 0.014 mg/kg.

12. References

[1] http://www.quechers.com/ or Anastassiades et al., J. AOAC Int., vol. 86, no. 2, p. 417, 2003.

[2] The Composition of Foods – fourth edition by Erling Saxholt, Gyldendals, 1996.

[3] Poulsen, M.E., Granby, K. (2000): Validation of a multiresidue method for analysis of pesticides in fruit, vegetables and cereals by GC/MS iontrap system. In Principle and Practices of Method Validation, edited by A. Fajgelj and A Ambrus. Special Publication No 256 from The Royal Society of Chemistry. ISBN 0-85404-783-2.

[4] 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.

[5] W. Horwitz, Anal. Chem., 1982; 54, 76A.

[6] Quality Control Procedures for Pesticide Residue Analysis- Guidelines for Residues Monitoring in the European Union, SANCO/10232/2006, 24/March/2006, European Commission, Brussels, 2006.

Appendix 1 - Summary of statistical values

Summary of statistical data based on data obtained in connection to the validation of 83 pesticides, isomers and degradation products in cereals using the QuEChERS method in accordance to CEN/TC 275/WG 4 N 0204. Data in italics and bold have not met the acceptance criteria.

Fortification level (mg/kg)		0.011	0.022	0.217	LOQ
Aclonifen	RSD_r, %	15	8	3	0,014
	RSD_R, %	***32***	14	8	
	Recovery,%	74	82	98	
Acrinathrin	RSD_r, %	5	14	5	0.016
	RSD_R, %	26	23	9	
	Recovery,%	95	93	84	
Benalaxyl	RSD_r, %	12	4	5	0.092
	RSD_R, %	20	9	7	
	Recovery,%	***153***	***131***	99	
Bifenthrin	RSD_r, %	***46***	12	1	0.024
	RSD_R, %	***33***	17	11	
	Recovery,%	***125***	108	94	
Bitertanol	RSD_r, %	14	24	7	0.012
	RSD_R, %	22	18	10	
	Recovery,%	90	95	102	
Bromophos-ethyl	RSD_r, %	10	9	4	0.022
	RSD_R, %	***40***	20	11	
	Recovery,%	94	92	93	
Bromopropylate	RSD_r, %	12	4	6	0.018
	RSD_R, %	26	19	11	
	Recovery,%	111	104	94	
Carbofenthion	RSD_r, %	16	15	5	0.016
	RSD_R, %	21	29	12	
	Recovery,%	122	102	103	
Carbofuran	RSD_r, %	***34***	17	3	0.026
	RSD_R, %	***92***	17	5	
	Recovery,%	***181***	114	109	
Chlorfenvinphos	RSD_r, %	9	5	2	0.018
	RSD_R, %	27	13	7	
	Recovery,%	***118***	113	105	
Chlorobenzilate	RSD_r, %	6	3	2	0.010

Fortification level (mg/kg)		0.011	0.022	0.217	LOQ
	RSD_R, %	16	14	6	
	Recovery,%	104	104	99	
Chloropropylate	RSD_r, %	9	3	2	0.012
	RSD_R, %	18	14	6	
	Recovery,%	102	104	99	
Chlorpyriphos	RSD_r, %	***32***	***44***	5	0.22
	RSD_R, %	***42***	***43***	19	
	Recovery,%	***132***	111	92	
Chlorpyriphos-methyl	RSD_r, %	7	6	5	0.024
	RSD_R, %	31	17	6	
	Recovery,%	***142***	117	101	
Chlorthal-dimethyl	RSD_r, %	14	16	2	0.014
	RSD_R, %	27	24	31	
	Recovery,%	89	89	89	
Cyprodinil	RSD_r, %	9	6	2	0.006
	RSD_R, %	9	9	7	
	Recovery,%	93	96	97	
Dialifos	RSD_r, %	9	9	9	0.014
	RSD_R, %	20	8	10	
	Recovery,%	110	111	104	
Diazinon	RSD_r, %	6	14	5	0.084
	RSD_R, %	26	18	7	
	Recovery,%	***163***	130	101	
Diclofenthion	RSD_r, %	11	12	6	0.006
	RSD_R, %	11	12	6	
	Recovery,%	95	92	96	
Diethofencarb	RSD_r, %	7	6	23	0.006
	RSD_R, %	8	8	34	
	Recovery,%	103	102	92	
Dioxathion	RSD_r, %	***27***	7	3	0.026
	RSD_R, %	29	20	9	
	Recovery,%	85	103	100	
Ethion	RSD_r, %	14	9	5	0.008
	RSD_R, %	13	10	6	
	Recovery,%	94	100	102	
Etrimfos	RSD_r, %	8	12	5	0.156

Fortification level (mg/kg)		0.011	0.022	0.217	LOQ
	RSD_R, %	29	23	12	
	Recovery,%	***192***	***143***	102	
Fenarimol	RSD_r, %	27	11	8	0.024
	RSD_R, %	29	16	9	
	Recovery,%	***128***	113	100	
Fenchlorphos	RSD_r, %	7	7	3	0.028
	RSD_R, %	***38***	19	5	
	Recovery,%	***141***	116	98	
Fenitrothion	RSD_r, %	6	5	5	0.014
	RSD_R, %	17	9	6	
	Recovery,%	***142***	118	104	
Fenoxaprop-p-ethyl	RSD_r, %	14	8	2	0.008
	RSD_R, %	12	12	5	
	Recovery,%	109	98	98	
Fenpropathrin	RSD_r, %	10	19	4	0.014
	RSD_R, %	19	24	8	
	Recovery,%	109	100	100	
Fenpropimorph	RSD_r, %	8	6	3	0.024
	RSD_R, %	20	17	11	
	Recovery,%	***139***	112	104	
Flusilazole	RSD_r, %	8	7	5	0.008
	RSD_R, %	11	9	8	
	Recovery,%	105	102	103	
Fonofos	RSD_r, %	24	14	9	0.014
	RSD_R, %	22	21	11	
	Recovery,%	91	94	96	
Furathiocarb	RSD_r, %	5	21	5	0.010
	RSD_R, %	14	15	9	
	Recovery,%	112	111	115	
Heptachlor	RSD_r, %	8	17	10	0.146
	RSD_R, %	28	25	11	
	Recovery,%	***195***	***150***	104	
Isofenphos	RSD_r, %	12	6	3	0.022
	RSD_R, %	27	15	5	
	Recovery,%	***119***	118	109	
Kresoxim-methyl	RSD_r, %	15	3	3	0.010

Fortification level (mg/kg)		0.011	0.022	0.217	LOQ
	RSD_R, %	13	8	5	
	Recovery,%	109	103	105	
Methidathion	RSD_r, %	16	19	6	0.114
	RSD_R, %	***44***	***41***	8	
	Recovery,%	***167***	***132***	110	
Molinate	RSD_r, %	19	11	15	0.22
	RSD_R, %	***34***	16	19	
	Recovery,%	106	***121***	90	
Myclobutanil	RSD_r, %	11	56	34	0.042
	RSD_R, %	14	50	57	
	Recovery,%	***124***	114	90	
Oxadixyl	RSD_r, %	23	4	2	0.018
	RSD_R, %	29	27	55	
	Recovery,%	95	99	90	
Parathion-methyl	RSD_r, %	10	10	4	0.066
	RSD_R, %	15	9	5	
	Recovery,%	***171***	***132***	106	
Penconazole	RSD_r, %	9	8	3	0.006
	RSD_R, %	9	11	7	
	Recovery,%	102	100	100	
Pendimethalin	RSD_r, %	17	6	2	0.008
	RSD_R, %	16	9	7	
	Recovery,%	83	86	97	
Phenthoat	RSD_r, %	8	8	4	0.018
	RSD_R, %	22	13	5	
	Recovery,%	***132***	114	105	
Phorat	RSD_r, %	7	9	7	0.102
	RSD_R, %	***35***	***30***	8	
	Recovery,%	***171***	***129***	96	
Phosalone	RSD_r, %	15	16	6	0.094
	RSD_R, %	***56***	27	7	
	Recovery,%	***190***	***146***	107	
Phosmet	RSD_r, %	***52***	20	4	0.24
	RSD_R, %	***61***	22	17	
	Recovery,%	***171***	***147***	110	
Pirimiphos-ethyl	RSD_r, %	8	5	3	0.024

Fortification level (mg/kg)		0.011	0.022	0.217	LOQ
	RSD_R, %	29	17	5	
	Recovery,%	***136***	115	103	
Pirimiphos-methyl	RSD_r, %	6	7	6	0.030
	RSD_R, %	***43***	21	5	
	Recovery,%	***126***	113	107	
Profenophos	RSD_r, %	***27***	7	7	0.026
	RSD_R, %	34	19	8	
	Recovery,%	102	109	105	
Propham	RSD_r, %	***27***	***26***	15	0.196
	RSD_R, %	***86***	***63***	13	
	Recovery,%	***209***	***180***	111	
Propyzamide	RSD_r, %	9	12	3	0.010
	RSD_R, %	15	13	4	
	Recovery,%	105	105	101	
Prothiofos	RSD_r, %	***51***	7	9	0.010
	RSD_R, %	***51***	7	13	
	Recovery,%	***122***	102	100	
Pyrimethanil	RSD_r, %	12	9	3	0.008
	RSD_R, %	12	11	5	
	Recovery,%	102	96	102	
Quinalphos	RSD_r, %	7	5	4	0.012
	RSD_R, %	14	13	6	
	Recovery,%	124	118	104	
Sulfotep	RSD_r, %	8	12	9	0.188
	RSD_R, %	***43***	22	14	
	Recovery,%	***169***	***135***	105	
Tebuconazole	RSD_r, %	***38***	11	4	0.112
	RSD_R. %	***54***	***63***	9	
	Recovery.%	***133***	***169***	104	
Tebufenpyrad	RSD_r. %	4	6	3	0.006
	RSD_R. %	10	12	7	
	Recovery.%	108	109	100	
Tetradifon	RSD_r. %	12	***43***	1	0.130
	RSD_R. %	***66***	***52***	10	
	Recovery.%	***245***	***139***	107	
Tetrasul	RSD_r. %	13	11	6	0.012

Fortification level (mg/kg)		0.011	0.022	0.217	LOQ
	RSD_R. %	22	13	11	
	Recovery.%	84	82	80	
Trichloronat	RSD_r. %	6	8	4	0.026
	RSD_R. %	***36***	18	10	
	Recovery.%	***127***	111	97	
Trifloxystrobin	RSD_r. %	14	17	4	0.012
	RSD_R. %	17	13	9	
	Recovery.%	116	110	108	
Trifluralin	RSD_r. %	13	14	12	0.008
	RSD_R. %	16	16	13	
	Recovery.%	86	92	95	
Vinclozolin	RSD_r. %	15	10	1	0.028
	RSD_R. %	***37***	19	4	
	Recovery.%	***130***	117	103	

Appendix 2 – List of ions used for MS quantification

Compound	Ions for quantification by MS			
Aclonifen	194	212	264	
Acrinathrin	181	208	289	
Amitraz	132	147	162	293
Benalaxyl	148	266	325	
Bifenthrin	165	166	181	
Binapacryl	83			
Biphenyl	152	153	154	
Bitertanol	170	171		
Bromophos-ethyl	303	331	359	
Bromopropylate	183	339	341	
Carbofenthion	157	199	342	
Carbofuran	149	164		
Chlorfenvinphos	267	269	323	
Chlorobenzilate	139	251	253	
Chloropropylate	139	251	253	
Chlorothalonil	264	266	268	
Chlorpyriphos	197	314		
Chlorpyriphos-methyl	286	288		
Chlorthal-dimethyl	303	332		
Cyprodinil	224	225		
Dialifos	208	210	357	
Diazinon	179	199	304	
Diclofenthion	223	251	279	
Dicofol	139	251		
Diethofencarb	196	225	267	
Dioxathion	197	270		
Ditalimfos	130	243	299	
Ethion	231	233	384	
Ethoxyquin	145	147	202	
Etridiazole	140	183	211	246
Etrimfos	181	277	292	
Fenamiphos	195	260	303	
Fenarimol	251	330		
Fenchlorphos	285	286	287	

Compound	Ions for quantification by MS			
Fenitrothion	260	277		
Fenoxaprop-p-ethyl	288	289		
Fenpropathrin	181	265		
Fenpropimorph	128			
Fludioxonil	127	154	182	248
Flusilazole	206	233	315	
Flutolanil	173	281	323	
Fonofos	246			
Furathiocarb	135	163	194	325
Heptachlor	272	274	337	
Hexachlorbenzen	249	282	284	286
Hexaconazole	175	214	231	
Iodofenphos	125	377	379	
Isofenphos	121	185	213	
ISTD-triphenylphosphate Har du brugt denne?	325	326		
Kresoxim-methyl	116	131	206	
Methidathion	85	145		
Molinate	98	126	154	
Myclobutanil	152	179	181	
Oxadixyl	132	163	233	
Parathion-ethyl	109	139	291	
Parathion-methyl	125	246	263	
Penconazole	248	250		
Pendimethalin	162	191	252	
Phenthoat	246	274		
Phenylphenol-2	141	169	170	
Phorat	75	231	260	
Phosalone	182	184	367	
Phosmet	160	161		
Pirimiphos-ethyl	168	318	333	
Pirimiphos-methyl	276	290	305	
Profenophos	337	339		
Propargite	173	201	350	
Propham	93	137	179	
Propyzamide	173	175	255	
Prothiofos	239	267	309	
Pyridaben	147	309	311	364

Compound	Ions for quantification by MS				
Pyrimethanil	198	199			
Quinalphos	146	156	157	298	
Spiroxamine	100	126	144	198	282
Sulfotep	266	294	322		
Tebuconazole	125	250	252		
Tebufenpyrad	171	276	318	333	
Tetradifon	159	229	356		
Tetrasul	252	254	324		
Tolylfluanid	137	181	238		
Trichloronat	269	297	299		
Trifloxystrobin	116	131	190		
Trifluralin	264	306	335		
Vinclozolin	198	212	214	285	

Appendix 3 – Examples of chromatograms obtained by GC-MS analysis

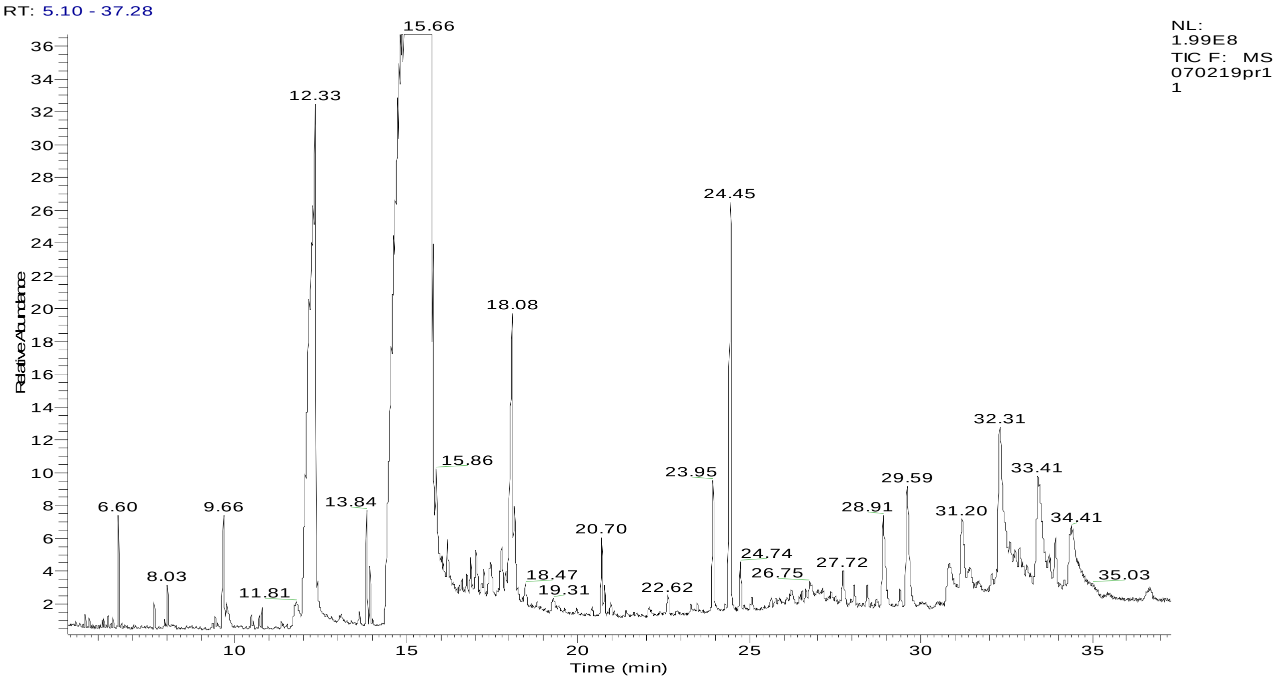

Figure 2. Cromatogram of a rice sample fortified with 0.022 mg/kg.

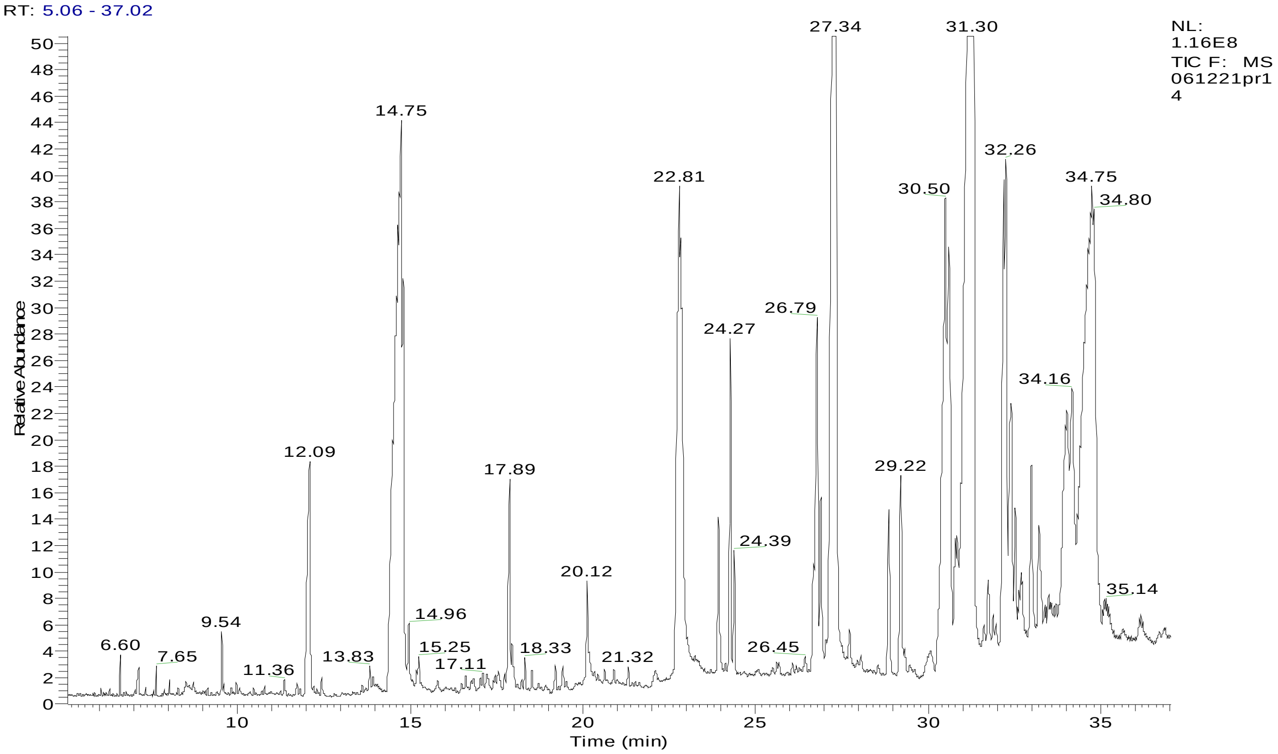

Figure 3. Cromatogram of a rye sample fortified with 0.022 mg/kg