Technical Note: 10239

Multi-residue Pesticide Analysis in Rice by a Modified QuEChERS Extraction and Ion Trap GC/MS^n Analysis

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Key Words

- ITQ 700
- Food Safety
- GC/MS^n
- Pesticide Analysis
- QuEChERS

Introduction

Recently formulated pesticides are quite different in their physical properties from their predecessors such as 4,4'-DDT. Most recently formulated pesticides are smaller in molecular weight and designed to break down rapidly in the environment. Therefore, to successfully identify and quantify these compounds in foods, careful consideration must be placed on the sample preparation for extraction and the instrument parameters for analysis. This study covers preparation of extracts and optimization of analytical parameters for injection, separation, and detection.

The determination of pesticides in fruits and vegetables has been simplified by a QuEChERS (**Qu**ick, **E**asy, **C**heap, **E**ffective, **R**ugged and **S**afe) extraction method, published recently as AOAC Method 2007.01.[1] The sample preparation is simplified by using a single-step buffered acetonitrile (MeCN) extraction and liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate ($MgSO_4$).[1] Analysis was performed by gas chromatography/tandem mass spectrometry (GC/MS^n) on the Thermo Scientific ITQ 700 GC-ion trap mass spectrometer.

The study determined the linear ranges, quantitation limits and detection limits for a list of pesticides that are commonly used on rice crops. A splitless injection of 33 pesticides was made with detection in electron ionization (EI) MS/MS. Since the extracts are prepared in MeCN, a solvent exchange was made to hexane/acetone (9:1) prior to conventional splitless injection.[2] Once the calibration curve was constructed, multiple matrix spikes were analyzed at a levels of 160, 320, or 480 ng/g (ppb) and low level spikes at 16, 32, 40, 80, or 120 ng/g (ppb) to verify the precision and accuracy of the analytical method.

Experimental Conditions

The sample preparation involves careful homogenization of the sample. Extraction solvents must be buffered and the powdered reagents measured at appropriate amounts for the size of sample prepared. Careful addition of the reagents must be taken since some reagents cause an exothermic reaction when mixed with water, which can adversely affect the recoveries of target compounds. The recommended consumables required for sample preparation and analysis were rigorously tested (Table 1). A list of the pesticides to be studied was created that would address

all of the various functional groups and different physical properties of most pesticides. A surge splitless injection was made into a Thermo Scientific TRACE TR-527 35% diphenyl/65% dimethyl polysiloxane column, (0.25 mm x 30 meter, and a film thickness of 0.25 µm with a 5 m guard column).

Item Descriptions
TRACE TR-527 35% diphenyl/65% dimethyl polysiloxane column, 0.25 mm x 30 meter, 0.25 µm w/5 m guard column
5 mm ID x 105 mm L liner (pk of 5)
10 µL syringe
Septa (pk of 50)
Liner graphite seal (pk of 10)
Ion volume - EI open
Ion volume holder
Graphite ferrule 0.1-0.25 (pk of 10)
Ferrule 0.4 mm ID 1/16 G/V (pk of 10)
Blank vespel ferrule for MS interface (pk of 10)
2 mL amber glass vial, silanized glass, with write-on patch (100/pk)
Blue cap with ivory PTFE/red rubber seal (100/pk)
Acetonitrile analytical grade (4L)
Hexane GC Resolv* (4L)
Acetone GC Resolv* (4L)
Organic bottle top dispenser
HPLC grade glacial acetic acid
50 mL Nalgene FEP centrifuge tubes (pk of 2)
Clean up tube: 15 mL tubes ENVIRO 900 mg $MgSO_4$, 300 mg PSA 150 mg C18 (pk of 50)
50 mL PP Tubes 6 g $MgSO_4$, 1.5 g $CH_3CHOONa$ (anhydrous) (pk of 250)
Clean up tube: 2 mL tubes 150 mg $MgSO_4$, 50 mg PSA (pk of 100)

Table 1: Consumables for QuEChERS Sample Prep and Analysis

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Sample Extraction and Clean Up

The QuEChERS sample prep procedure consists of the steps shown in Figure 1. There are three parts: the extraction, the clean-up, and solvent exchange. The solvent exchange provides a final solvent that is more amenable to splitless injection. Care must be taken to adequately and thoroughly homogenize the sample. When analyzing grains such as rice, water must be added during the homogenization step and taken into consideration in the final calculations of spikes and standards. To perform liquid-liquid extraction requires water. Also, the water helps mix the rice during the homogenization step.

A thoroughly homogenized 15 g sample of rice was weighed into this extraction tube. Then 15 mL of 1% glacial acetic acid MeCN extraction solvent was poured into the tube on top of the sample. The surrogate and the pesticide solutions were spiked into this MeCN layer for the method validation (MVD) and method detection limit (MDL) samples.

The tube was capped and vortexed for 30 seconds. The cap was removed and the powder reagents were poured slowly into the MeCN layer. The cap was tightened securely on the 50 mL extraction tube, and was vortexed for 30 seconds until all of the powder reagents were mixed with the liquid layers. The tube was placed on a mechanical shaker for 5 minutes and then centrifuged for 5 minutes at 3000 rpm. Next, 11 mL of the top MeCN layer was removed and transferred to a 15 mL clean-up tube. This tube was capped and vortexed for 30 seconds and centrifuged for 5 minutes at 3000 rpm. A 5 mL aliquot of the top layer was transferred into a clean test tube for solvent exchange.

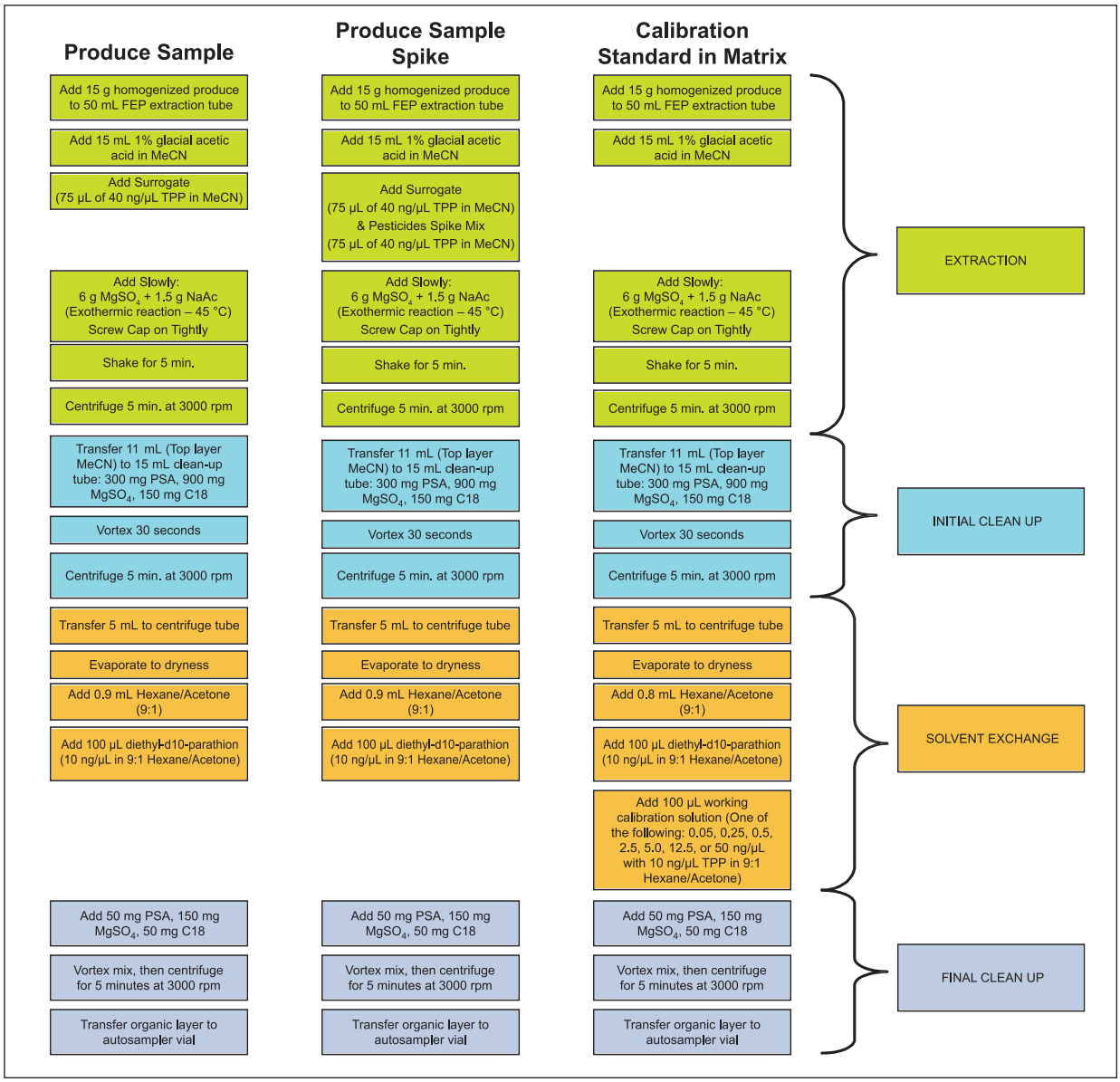


Figure 1: Flow diagram of QuEChERS sample preparation procedure

Solvent Exchange

The 5 mL aliquot of cleaned up extract was blown down to dryness with a gentle stream of nitrogen at 40 °C in about one hour. Care was taken to remove the tube immediately when dried. A 900 µL aliquot of hexane/acetone (9:1) was added and 100 µL of the internal standard, d10-parathion, was spiked into the organic solution. The tube was capped and vortexed for 15 seconds. The 1 mL of extract was transferred to a 2 mL clean-up tube, capped tightly, and vortexed for 30 seconds. After centrifuging for 5 minutes at 3000 rpm, 200 µL of the clear extract was transferred to an autosampler vial with a small glass insert for analysis on the ITQ 700 system. The individual calibration levels were spiked into each extract for the calibration curve in matrix before the final cleanup step (Figure 1).

Injection

The ITQ 700 is paired with the Thermo Scientific FOCUS GC gas chromatograph, which is a single-channel GC with a standard split/splitless (SSL) injection port. The SSL inlet temperature was set to 250 °C. A 5 mm i.d. splitless liner with a volume of 1.6 mL was selected for the surged pressure injection. For the surge splitless injection, the inlet pressure was held at an elevated pressure of 250 kPa for the 0.5 minute injection (splitless) time. This technique reduces the vapor cloud of a 2 µL injection from 0.37 mL to 0.19 mL. At an elevated injection flow rate of 4.6 mL/ min., the liner was swept several times during injection. The target compounds moved through the inlet rapidly thus reducing the time to interact with the inside walls of the liner. This minimized the amount of breakdown of the more fragile pesticides.

A Performance Solution consisting of endrin and 4,4'-DDT was analyzed as a daily check to determine system activity. The analysis of endrin, DDT, and their breakdown products as part of daily quality control can alert the analyst that the system has developed active sites and maintenance is needed. Without performing a breakdown analysis the laboratory may need to continually maintain the equipment and replace consumables, even when it may not be needed. Monitoring breakdown can decrease the cost of running the analysis and save significant amounts of time. Endrin breakdown is determined by adding up the response for the two breakdown products: endrin aldehyde and endrin ketone and dividing by the total response for the breakdown products and endrin in percent. The breakdown products of DDT are DDE and DDD and are calculated similarly. The breakdown check results showed < 5 % breakdown for both compounds on a daily basis (Figure 2). For routine use the liner would be changed when the breakdown of either compound reaches > 20%. The injection port liner tested showed very good results over a long period of time without the need for maintenance.

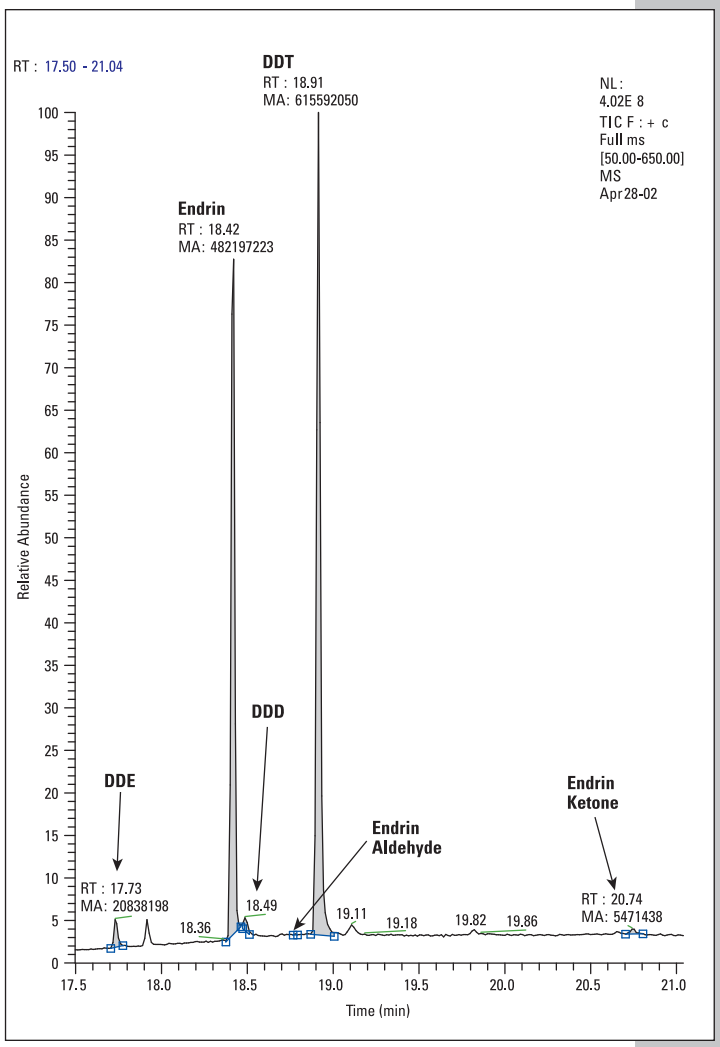


Figure 2: Endrin and DDT breakdown analysis, showing < 5% breakdown

Separation

Chromatographic separation was achieved by using a 35% diphenyl/65% dimethyl polysiloxane column (0.25 mm x 30 meter, and a film thickness of 0.25 µm with a 5m guard column). This column was chosen to provide sufficient resolution of the more polar compounds. The oven was programmed as follows:

Initial Temp: 40 °C, 1.5 min., 25 °C/min. to 150 °C, 0.0 min, 5 °C/min. to 200 °C, 7.5 min., 25 °C/min. to 290 °C with a final hold time of 12 min. and a constant column flow rate of 1 mL/min.

The entire set of instrument parameters is listed in Table 2.

AS 3000 II Autosampler	
Sample Volume	2 µL
Plunger Strokes	5
Viscous Sample	No
Sampling Depth in Vial	Bottom
Injection Depth	Standard
Pre-inj Dwell Time	0
Post-inject Dwell Time	0
Pre-inject Solvent Wash Vial Position	A + B
Pre-inject Solvent Wash Cycles	3
Sample Rinses	3
Post-inject Solvent	A
Post-inject Solvent Cycles	3

FOCUS GC	
Column	TRACE TR-527 w/ guard column 0.25 mm x 30 meter, 0.25 µm
Column Constant Flow	1 mL/min.
Oven Program	40°, 1.5 min., 25°/min.; 150°, 0.0 min., 5°/min., 200°, 7.5 min., 25°/min., 290°, 12 min.
S/SL Temperature	250 °C
S/SL Mode	Splitless with Surge Pressure
Surge Pressure	250 kPa
Inject Time	0.5 min.
Split Flow	50 mL/min.
Transferline Temperature	290 °C

ITQ Mass Spectrometer	
Damping Gas Flow	2
Source Temperature	250 °C
Ion Volume	EI
Emission Current	250 µA
Detector Gain	3 (1367 V)
Lens 1	-25V
Lens 3	-25V
Gate Lens On	-100
Gate Lens Off	100
Electron Lens On	15V
Electron Lens Off	85
Electron Energy	-70eV
Trap Offset	-10
Waveforms	Off

Table 2: Selected instrument parameters for the ITQ 700 system

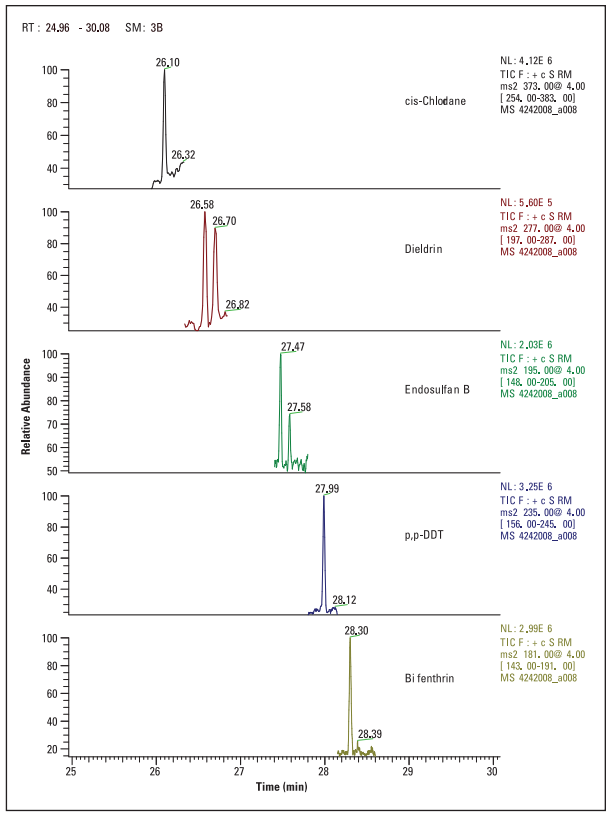


Figure 3: MS/MS scan of 160 ng/g in rice matrix

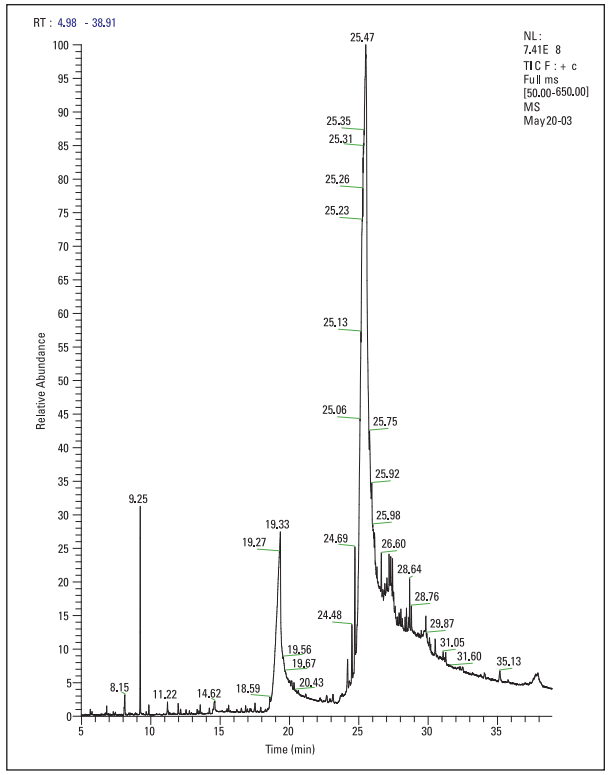


Figure 4: Full scan chromatogram of 160 ng/g of pesticides in rice

Detection

The detection of the pesticides was performed using the ITQ 700 ion trap mass spectrometer with optional MS^n mode. This scanning mode offers enhanced selectivity over either full scan or selected ion monitoring (SIM). In SIM at the elution time of each pesticide, the ratio of the intensity of matrix ions increases exponentially versus that of the pesticide ions as the concentration of the pesticide approaches the detection limit, decreasing the accuracy at lower levels. The ITQ 700 operated in the MS^n mode performs tandem MS functions by injecting ions into the ion trap and destabilizing matrix ions, isolating only the pesticide ion. These pesticide ions are given sufficient energy to further fragment and are then scanned. This process provides the product ion spectrum. This is done by setting up a stable field for the pesticide precursor ion. Once the precursor ion is isolated from the matrix ions, Collision Induced Dissociation (CID) energy is applied to fragment it into its respective product ions. Finally these unique product ions are scanned out to generate the product ion spectrum. Because of the elimination of matrix interferences, this process produces more accurate results at the lower levels. The MS^n parameters for the ITQ 700 are listed in Table 3. Figures 3 and 4 show a comparison between a Full Scan and MS^n TIC.

Compound	RT (Minutes)	Precursor *(m/z)*	Width (amu)	Collision Energy (Volts)	Max. Excitation Energy (q)	Range *(m/z)*	Product Ion *(m/z)*	Qualifiers *(m/z)*
Dichlorvos	8.49	185	1	3	0.225	53-195	93	131, 109, 170, 63
Molinate	13.04	126	2	3	0.3	45-136	98	83, 55, 82, 81
Trifluralin	13.33	264	2	3	0.225	150-274	206	188, 160, 171, 177
Tebuthiuron	13.35	156	1	3	0.225	52-166	89	74, 62, 87, 125
Propachlor	14.47	120	2	4	0.3	67-130	92	91, 103, 77, 93
Phorate	15.73	231	2	3	0.225	165-241	203	175, 185
Propyzamide (Pronamide)	16.79	173	2	3	0.225	135-183	145	146
Diazanon	17.51	179	1	4	0.225	86-189	137	164, 138, 161, 96
Gamma BHC (Lindane)	17.89	219	4	3	0.225	171-229	181	183, 182, 184
b-BHC	18.34	181	2	3	0.225	135-191	145	146
Heptachlor	19.97	272	2	3	0.225	225-282	237	235, 268
Chlorothalanil	20.09	266	1	5	0.225	193-276	231	203, 205, 233, 229
d-BHC	20.35	181	2	3	0.225	135-191	145	146
Aldrin	22.12	263	1	5	0.225	217-273	229	228, 227, 230, 249
Metalaxyl	22.79	160	1	3	0.225	120-170	145	130, 132
Terbutryn	23.29	185	3	3	0.225	142-195	170	157, 152
Metolachlor	23.57	162	2	4	0.225	110-172	133	134, 120, 144, 147
Metribuzin	23.68	198	2	4	0.225	93-208	151	103, 110, 153, 128
Thiobencarb	24.09	100	3	5	0.45	52-110	72	71, 73, 99, 62
Sevin (Carbaryl)	24.12	144	1	3	0.3	105-154	116	115
Dursban (Chlorpyrifos)	24.14	314	5	3	0.225	248-324	286	258, 287, 288, 285
Malathion	24.31	173	3	4	0.225	125-183	136	145, 137, 138, 135
Parathion-d10	24.34	301	2	3	0.225	105-311	269	147, 115, 148, 271
Methiocarb	24.4	168	1	3	0.225	99-178	153	109
Terbufos (Sulfone)	26.01	199	7	3	0.225	133-209	171	172, 153, 143, 173
cis-Chlordane	26.06	373	5	4	0.225	254-383	301	337, 299, 264, 335
Dieldrin	26.67	277	3	4	0.225	197-287	241	242, 239, 207, 217
Endrin	27.25	263	1	5	0.225	183-273	228	230, 226, 229, 193
Endosulfan B	27.56	195	2	4	0.225	148-205	159	160, 158
p,p-DDT	27.97	235	4	4	0.225	156-245	166	200,199,201, 202
Bifenthrin	28.3	181	7	4	0.225	143-191	166	165, 167, 178, 153
Methoxychlor	29.44	227	7	5	0.225	175-237	212	195, 196, 185, 197
trans-Permethrin	31.2	183	3	4	0.225	143-193	168	165, 155, 153, 181
Fluridone	37.32	328	2	6	0.225	249-338	259	288, 313, 308, 268

Table 3: MS/MS parameters for pesticide analysis

Compound	(R^2)	Compound	(R^2)
Dichlorvos	0.9999	Metribuzin	1.0000
Molinate	0.9997	Thiobencarb	1.0000
Trifluralin	0.9983	Sevin (Carbaryl)	0.9982
Tebuthiuron	0.9995	Dursban (Chlorpyrifos)	0.9979
Propachlor	0.9995	Malathion	0.9996
Phorate	0.9996	Methiocarb	0.9999
Propyzamide (Pronamide)	0.9983	Terbufos Sulfone	0.9997
Diazanon	1.0000	*cis*-Chlordane	0.9995
Gamma BHC	0.9999	Dieldrin	0.9971
b-BHC	0.9989	Endrin	0.9989
Heptachlor	0.9997	Endosulfan B	0.9998
Chlorothalinil	0.9997	p,p-DDT	0.9983
d-BHC	0.9988	Bifenthrin	1.0000
Aldrin	0.9988	Methoxychlor	0.9986
Metalaxyl	0.9992	*trans*-Permethrin	0.9998
Terbutryn	0.9993		
Metolachlor	0.9996	**Average**	**0.9993**

Table 4: Calibration curve results

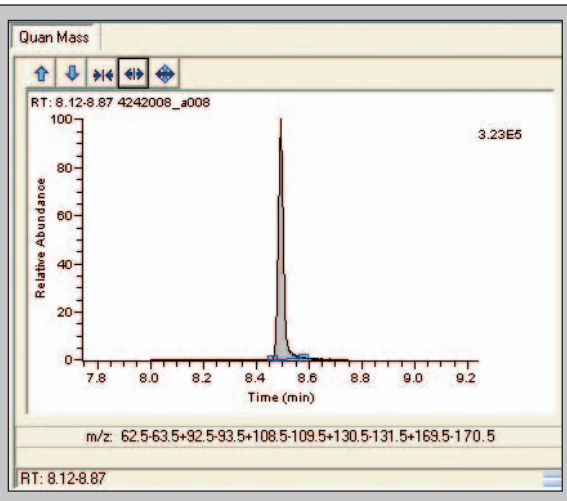
Results and Discussion

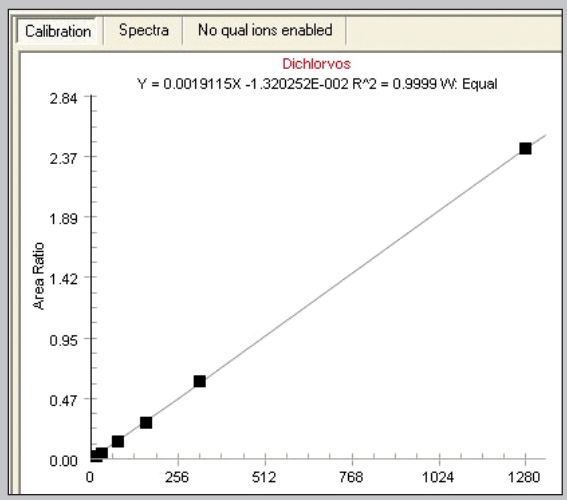
Linearity

The calibration curve was spiked into the rice matrix. Levels ranged from 1 ng/g to 1200 ng/g, depending on the compound and its MRL in rice. The linearity for all compounds was $R^2 > 0.995$. The results of the linearity are shown in Table 4. Figures 5 and 6 are two examples of calibration curves.

Limits of Detection and Quantitation

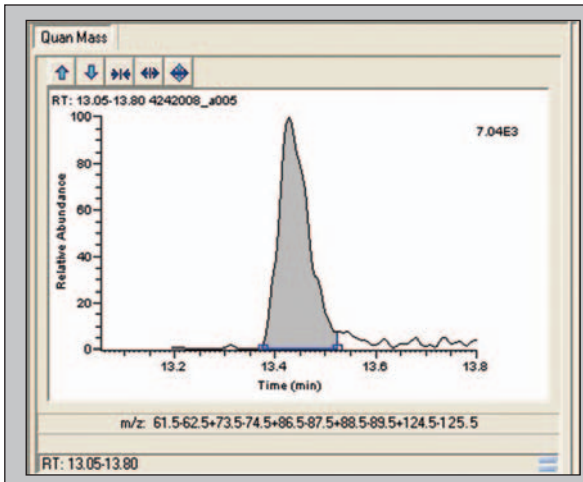
The actual limit of detection (LOD) and limit of quantitation (LOQ) were determined by preparing matrix spikes at a level near or below the MRL. Concentrations of 16, 32, 40, 80, and 120 ng/g were analyzed in seven matrix samples, and the LOD and LOQ were calculated from these results by multiplying the standard deviation of the calculated amounts by 3 and 10 respectively. The results are shown in Table 5.

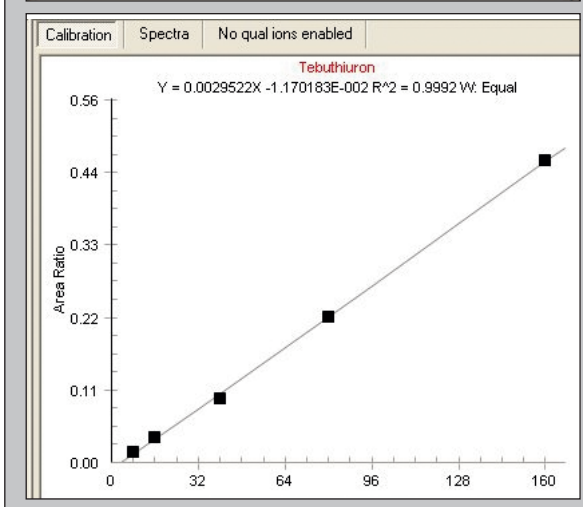




ng/g (ppb) Specified Amount	ng/g (ppb) Calculated Amount	% D	Lowest MRL Japan
16	19	-20.0	
32	31	3.1	
80	79	1.0	
160	154	3.6	200
320	325	-1.6	
1280	1280	0.0	

Figure 5: MS/MS Calibration curve for dichlorvos, from 16 to 1280 ppb





ng/g (ppb) Specified Amount	ng/g (ppb) Calculated Amount	% D	Lowest MRL Japan
8	9	-12.5	
16	17	-3.8	20
40	38	6.3	
80	81	-0.9	
160	160	-0.1	

Figure 6: MS/MS calibration curve for tebuthiuron, from 8 to 160 ppb

Component	Ave. Conc. (ng/g)	Std. Dev.	% RSD	LOD (ng/g)	LOQ (ng/g)	Japan[1] MRL (ng/g)	US-EPA[2] MRL (ng/g)	EU[3] MRL (ng/g)	EU[3] LOD[3]	WHO[4] MRL (ng/g)
Dichlorvos	60	3.6	6.1	11	36	200		2000		
Molinate	40	1.1	2.9	4	11	100				
Trifluralin	41	2.6	6.3	8	26	50				
Tebuthiuron	33	2.8	8.8	9	28	20				
Propachlor	34	2.6	7.5	8	26	50				
Phorate	45	1.5	3.3	5	15	50		50	50	
Propyzamide (Pronamide)	31	2.6	8.2	8	26	20		20	20	
Diazanon	86	2.4	2.8	8	24	100		20	20	
Gamma BHC	85	1.6	1.8	5	16	300		10	10	
b-BHC	48	1.1	2.4	4	11	200		10	10	
Heptachlor	14	2.0	14.4	6	20	20		10		
Chlorothalinil	27	1.4	5.4	4	14	100		10		
d-BHC	43	1.1	2.6	3	11	200		10	10	
Aldrin	44	1.1	2.5	3	11	ND				
Metalaxyl	30	4.7	15.4	15	47	100		50	50	
Terbutryn	51	1.9	3.7	6	19	100				
Metolachlor	38	1.4	3.7	4	14	100	100			
Metribuzin	46	3.7	8.1	12	37	50				
Thiobencarb	44	2.2	5.1	7	22	200	200			
Sevin (Carbaryl)	32	3.7	11.6	12	37	1000	5000			
Dursban (Chlorpyrifos)	113	2.9	2.6	9	29	100		50	50	500
Malathion	31	4.4	14.3	14	44	100	8000			
Methiocarb	26	2.4	9.2	8	24	50				
Terbufos Sulfone	36	1.4	3.8	4	14	5				
cis-Chlordane	18	1.6	9.1	5	16	20				
Dieldrin	45	4.4	9.8	14	44	ND				
Endrin	41	6.4	15.7	20	64	ND				
Endosulfan B	39	5.3	13.7	17	53	100				
p,p-DDT	44	2.3	5.2	7	23	200				
Bifenthrin	34	2.0	5.7	6	20	1000		50	50	
Methoxychlor	44	3.4	7.7	11	34	2000	2000			
trans-Permethrin	33	4.6	14.2	14	46	2000		50	50	
Fluridone	30	8.0	26.3	25	80	100				
Average			**7.9**	**9**	**29**					

1. CODEX alimentarius (www.codexalimentarius.net/mrls/pesticides/jsp/pest-q-e.jsp)

2. Japanese Food Chemical Research Foundation (www.m5.ws001.squarestart.ne.jp/foundation/search.html)

3. Informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC (5058/VI/98

4. 40CFR180 (www.access.gpo.gov/nara/cfr/waisidx_02/40cfr180_02.html)

Table 5: Comparison of LODs and LOQs vs MRLs

Method Validation Results

The method validation (MVD) calculations were performed using five matrix samples spiked at concentrations of 160, 320, or 480 ng/g per pesticide. Samples had an average of 98% recovery with an average % RSD of 5.9%. MVD results are shown in Table 6.

Conclusions

The Thermo Scientific ITQ 700 GC-ion trap MS was thoroughly evaluated and showed excellent accuracy at low concentrations of 33 pesticide residues analyzed in rice. Using the instrument's MS^n functionality allows the user to identify, confirm, and quantify in one analytical run. The injector demonstrated low endrin and DDT breakdown (< 5%) on a daily basis, proving that the system can analyze active compounds without the need for continual, expensive, and time-consuming maintenance. Calibration curves for the pesticides studied met a linear least squares calibration with a correlation coefficient of $R^2 > 0.995$ for all compounds. The Method Validation Study generated an average % RSD of 5.9% for five replicate analyses at 160, 320, or 480 ng/g and a calculated average LOD of 9 ng/g in rice based on 7 replicate analyses of 16, 32, 40, 80, and 160 ng/g.

Component	Average Conc	Theoretical Conc	% Difference	% RSD	% Recovery
Dichlorvos	272	320	-15.1	2.9	84.9
Molinate	158	160	-1.0	2.6	99.0
Trifluralin	207	160	29.3	7.5	129.3
Tebuthiuron	149	160	-7.1	10.5	92.9
Propachlor	170	160	6.4	4.0	106.4
Phorate	175	160	9.1	4.1	109.1
Propyzamide (Pronamide)	374	320	16.9	4.6	116.9
Diazanon	352	320	10.1	5.3	110.1
Gamma BHC	330	320	3.0	4.2	103.0
b-BHC	173	160	8.4	4.8	108.4
Heptachlor	178	160	11.1	6.6	111.1
Chlorothalinil	280	320	-12.6	5.7	87.4
d-BHC	152	160	-5.0	4.6	95.0
Aldrin	154	160	-3.5	4.8	96.5
Metalaxyl	177	160	10.4	6.9	110.4
Terbutryn	168	160	5.1	4.4	105.1
Metalochlor	167	160	4.7	4.2	104.7
Metribuzin	172	160	7.5	5.7	107.5
Thiobencarb	154	160	-4.0	4.2	96.0
Sevin (Carbaryl)	149	160	-6.7	6.4	93.3
Dursban (Chlorpyrifos)	487	480	1.5	4.0	101.5
Malathion	155	160	-2.9	7.0	97.1
Methiocarb	119	160	-25.5	4.6	74.6
Terbufos Sulfone	153	160	-4.5	4.5	95.5
cis-Chlordane	153	160	-4.3	6.3	95.7
Dieldrin	172	160	7.7	5.7	107.7
Endrin	161	160	0.5	5.8	100.5
Endosulfan B	157	160	-2.1	11.2	97.9
p,p-DDT	133	160	-16.9	8.2	83.1
Bifenthrin	150	160	-6.5	8.2	93.5
TPP (Surrogate)	161	200	-19.7	5.5	80.3
Methoxychlor	139	160	-13.4	6.8	86.6
trans-Permethrin	271	320	-15.3	8.9	84.7
Fluridone	115	160	-28.4	10.3	71.6
Average				**5.9**	**98.1**

Table 6: Results of method validation study

References

1. AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate, S. Lehotay, Journal of AOAC International Vol. 90, No. 2, (2007) 485–520
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