Technical Note: 10236

Quick Start-Up for a Modified QuEChERS Multi-residue Pesticide Analysis in Lettuce by GC/MS^n

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Key Words

- ITQ Series GC-Ion Trap MS
- Food Safety
- GC/MS^n
- Pesticide Residues
- 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, more careful consideration must be placed on the sample preparation for extraction and the instrument parameters for analysis. This study will cover the preparation of extracts and the optimization of the analytical parameters of the splitless injection, separation, and detection.

The determination of pesticides in fruits and vegetables has been simplified by a new sample preparation method, QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe), 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] This technical note describes the application of the QuEChERS sample preparation procedure to analysis by gas chromatography/ tandem mass spectrometry (GC/MS^n) on the Thermo Scientific ITQ 700™ GC-ion trap mass spectrometer.

The study was performed to determine the linear ranges, quantitation limits and detection limits for a list of pesticides that are commonly used on iceberg lettuce crops, prepared in matrix using the QuEChERS sample preparation guidelines. A splitless injection was made with detection in 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] Following construction of the calibration curve, multiple matrix spikes were analyzed at a high level of 50 ng/g and a low level spike of 5 ng/g to verify the precision and accuracy of the analytical method.

Experimental Conditions

During the method validation, several experiments were performed to determine the effect of minor modifications to the QuEChERS method which may impact the performance of the analysis in the laboratory. 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. 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. MS^n parameters were optimized using variable buffer gas, testing the isolation efficiency, and adjustment of the Collision Induced Dissociation (CID) voltage. A surge splitless injection was made into a 5% diphenyl/95% dimethyl polysiloxane column (0.25 mm x 30 meter, and a film thickness of 0.25 µm).

Item Descriptions
Thermo Scientific TRACE™ TR-5MS SQC (0.25 mm x 30 meter, 0.25 µm)
5 mm ID splitless injection port liner, 105 mm length (pk of 5)
10 µL syringe
Septa (pk of 50)
Graphite liner seal (pk of 10)
Open EI ion volume
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 vials, silanized, with write-on patch, (pk of 100)
Blue cap with Ivory PTFE/Red rubber seal, (pk of 100)
Acetonitrile analytical grade (4L)
Hexane GC Resolv* Grade (4L)
Acetone GC Resolv* Grade (4L)
Organic bottle top dispenser
HPLC grade glacial acetic acid
50 ml FEP centrifuge tubes
Initial clean-up tubes: 15 mL tube 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)
Final clean-up tube: 2 mL tube 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 two main parts: the extraction and the clean up. A third section was added for a solvent exchange to provide a final solvent that is more amenable to splitless injection. Care must be taken to adequately and thoroughly homogenize the sample.

During the extraction phase of the sample preparation, an observation was made that if the MeCN extract was poured into the $MgSO_4$, poor spike recoveries were observed. This is due to the exothermic reaction of the water in the sample and $MgSO_4$. Although many vendors offer the pre-measured powder reagents in a separate capped centrifuge tube, do not add the sample to these tubes. The reagents from these tubes should instead be added directly to the sample containing the acidified MeCN. Because of this, an empty 50 mL FEP extraction tube was included in the list of consumables for sample preparation. A thoroughly homogenized 15 g sample of iceberg lettuce 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 solution 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.

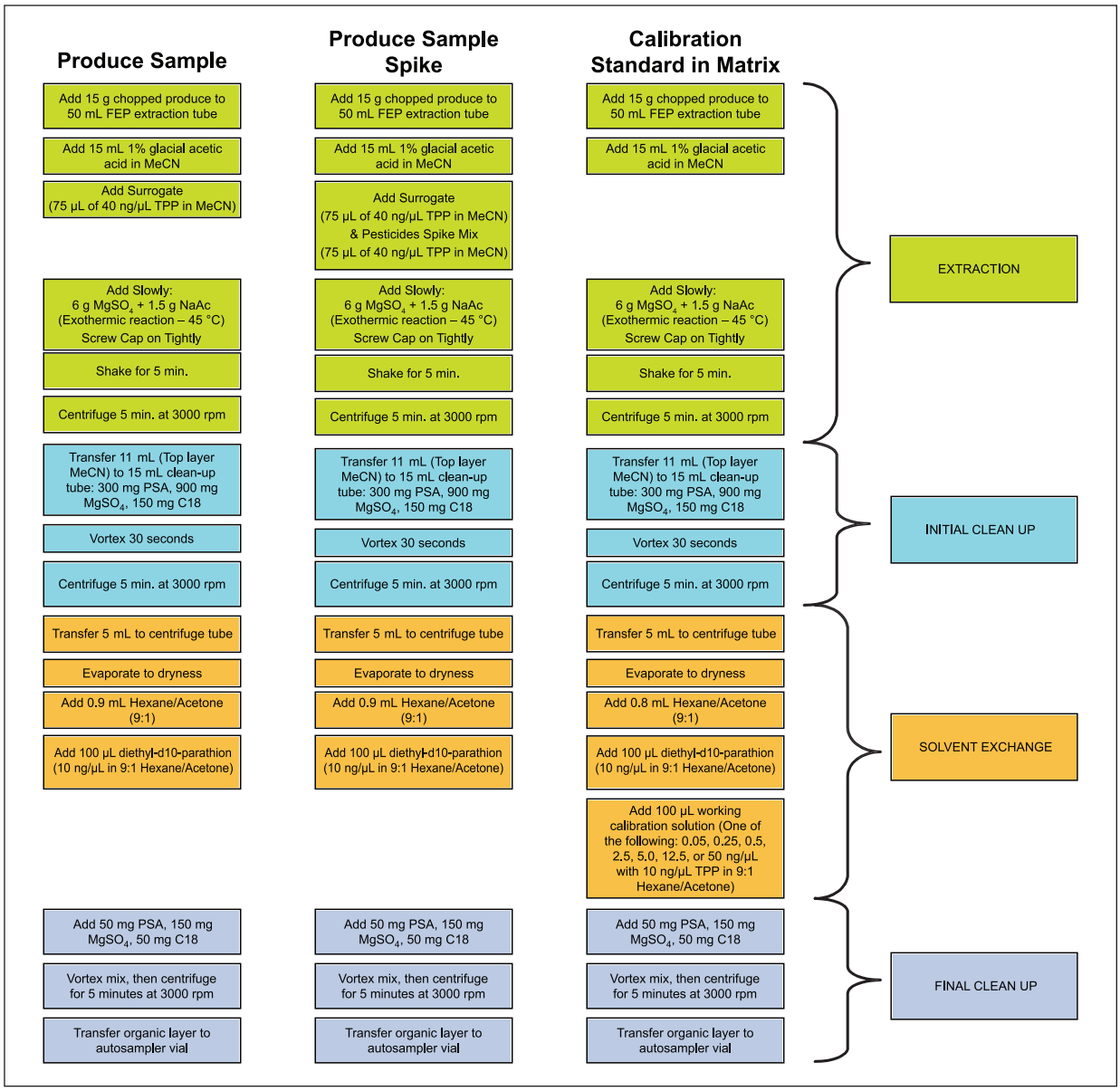

Figure 1: Flow Diagram of Modified QuEChERS Sample Prep

Solvent Exchange

The 5 mL aliquot of cleaned up extract was evaporated to dryness under a gentle stream of nitrogen at 40 °C (about one hour). Care was taken to remove the tube immediately to prevent over-drying. 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 light green clear extract was transferred to an autosampler vial with a small glass insert for injection on the ITQ 700. The individual calibration levels were spiked into each extract for the calibration curve in matrix (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 240 °C. A 5 mm ID 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 200 kPa for the 1.0 minute injection (splitless) time. This technique reduces the vapor cloud of a 2 µL injection from 0.72 mL to 0.54 mL. At an elevated injection flow rate of 1.8 mL/min., the liner was swept in less than a minute during injection. Using this technique, target compounds move through the inlet so rapidly they have less time to interact with the inside walls of the liner. This minimizes the amount of breakdown of the more fragile pesticides.

A Performance Solution consisting of DFTPP, endrin and 4,4'-DDT was analyzed as a daily check to determine system activity. The analysis of endrin and its 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 of endrin the laboratory may maintain the equipment and replace consumables, even when it may not be needed. Performing the breakdown test can decrease the cost of running the analysis and save a significant amount 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 check results showed < 10% endrin breakdown on a daily basis. For routine use the liner would be changed when the breakdown reaches > 20%. The injection port liner tested showed very good results over a long period of time without the need for maintenance (Figure 2).

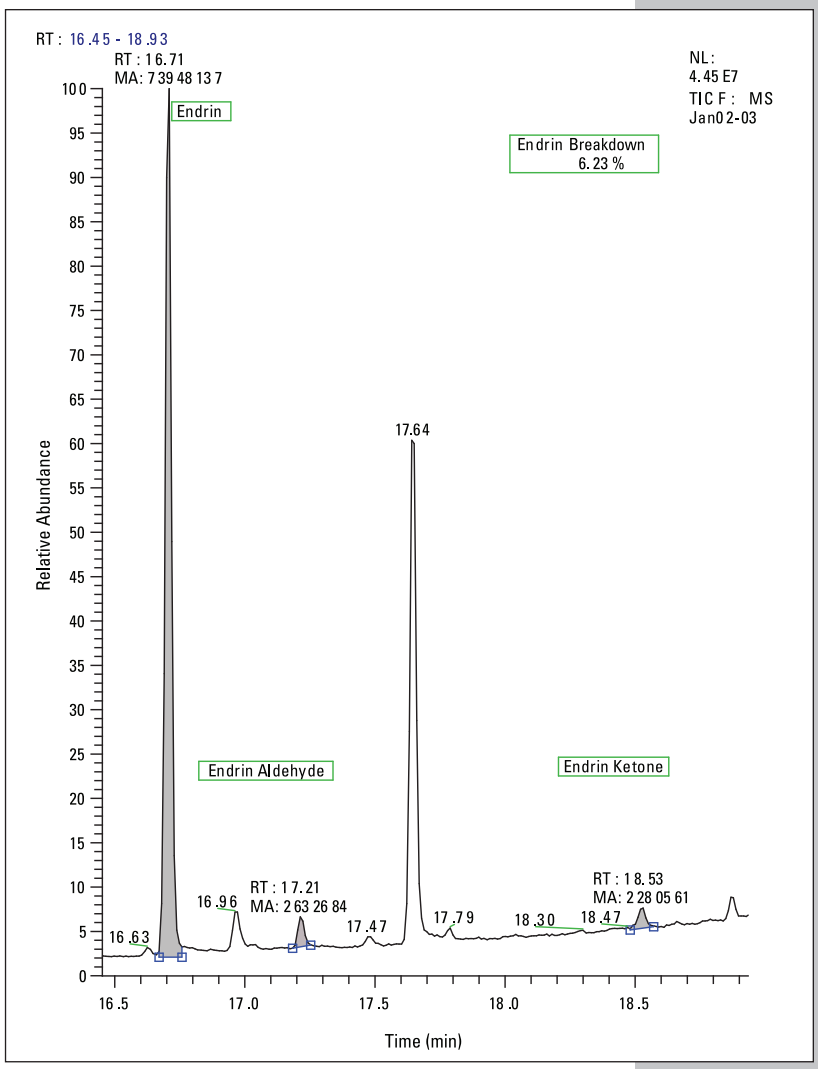

Figure 2: Endrin breakdown analysis, demonstrating that the system is free from excessive activity

AS 3000 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	TR-5MS SQC 0.25 mm x 30 m, 0.25 µm
Column Constant Flow	1 mL/min He
Oven Program	50 °C, 1.0 min, 25 °C/min;125 °C, 0.0 min, 10 °C/min, 300 °C, 7.5 min
SSL Temperature	240 °C
SSL Mode	Splitless with Surge Pressure
Surge Pressure	200 kPa
Splitless Duration	1.0 min
Split Flow	50 mL/min
Transferline Temperature	280 °C

ITQ 700 Mass Spectrometer	
Damping Gas Flow	2 mL/min He
Source Temperature	220 °C
Ion Volume	EI
Emission Current	250 µA
Detector Gain	3 (1306V)
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 autosampler, GC, and mass spectrometer

Separation

Chromatographic separation was achieved by using a 5% diphenyl/95% dimethyl polysiloxane column (0.25 mm x 30 meter, and a film thickness of 0.25 µm). This is a non-polar phase that works quite well for heavily chlorinated pesticides. Some interactions within the stationary phase showed a loss of certain pesticides at concentrations below 100 pg. The oven was programmed as follows: Initial Temp: 50 °C, 1.0 min, 25 °C/min to 125 °C; 10 °C/min to 300 °C with a final hold time of 7.5 min and a constant column flow rate of 1 mL/min. The entire set of instrument parameters is listed in Table 2.

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 scanning modes such as full scan and selected ion monitoring (SIM). In SIM at the elution time of each pesticide, the ratio of the intensity of matrix ions increases versus that of the pesticide ions as the concentration of the pesticide approaches the detection limit. This compromises the ability to positively identify the pesticides leading to false negatives.

The ITQ 700 operated in the MS^n mode performs a tandem MS scan by injecting ions into the ion trap and destabilizing matrix ions. Only ions of a specified pesticide mass are stored. These pesticide ions, known as precursors, are given sufficient energy and fragment through Collision Induced Dissociation (CID). Finally these unique product ions are scanned to generate the product ion spectrum. Because of the elimination of matrix interferences, this process produces more accurate and precise results at low levels. This makes lower limits of detection and quantitation possible. Confidence in the presence of a given pesticide is also improved since detecting product ions which result from the fragmentation of a specific precursor ion is more selective, and therefore less prone to interference, than a comparable full scan or SIM method. Figures 3 and 4 show a comparison of Full Scan and MS^n chromatograms.

Compound	Precursor *(m/z)*	Isolation Width (amu)	CID Voltage	q (Excitation Energy)	Product Ion Range *(m/z)*	Product Ion *(m/z)*	Qualifiers *(m/z)*
mevinphos	127	7	7	0.45	69-137	109	127, 79
dimethoate	125	7	3	0.3	52-89	79	62, 78
gamma BHC	219	4	1	0.3	171-228	217	218, 216, 181
diazanone	179	5	4	0.3	86-174	137	164, 138, 161, 96
chlorothalanil	266	1	4.7	0.45	193-243	229	231, 203, 205, 233
vinclozolin	212	5	4.5	0.3	162-189	172	177, 179, 176
metalaxyl	160	6	3.7	0.3	120-155	145	130, 144, 132
methiocarb	168	7	3.5	0.45	99-164	153	154, 109
dichlofluanid	167	6	3.3	0.3	113-145	124	135
cyprodinil	224	5	5.3	0.45	187-219	208	209, 207, 197
thiabendazole	201	5	3.5	0.45	164-211	174	201
folpet	260	5	2.5	0.225	120-270	232	130, 200, 260
imazalil	173	5	4	0.45	127-183	145	137, 173
endosulfan sulfate	387	3	2.5	0.225	241-299	289	253, 254, 251, 277
cis-permethrin	183	7	4	0.3	143-191	168	165, 155, 153, 181
trans-permethrin	183	7	4	0.3	143-191	168	165, 155, 153, 181

Table 3: ITQ 700 MS^n parameters

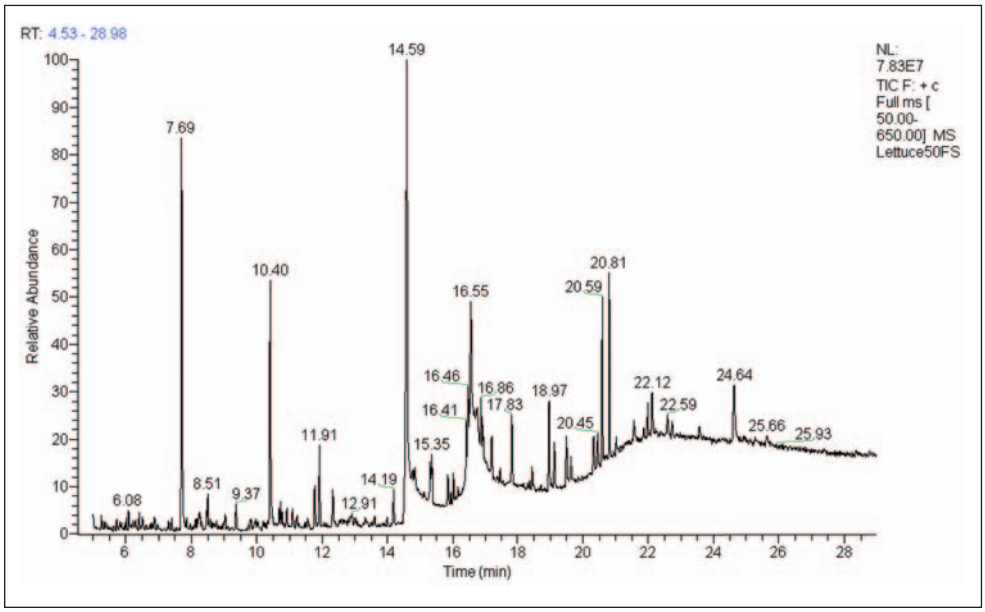

Figure 3: Full scan of 50 ng/g in lettuce matrix

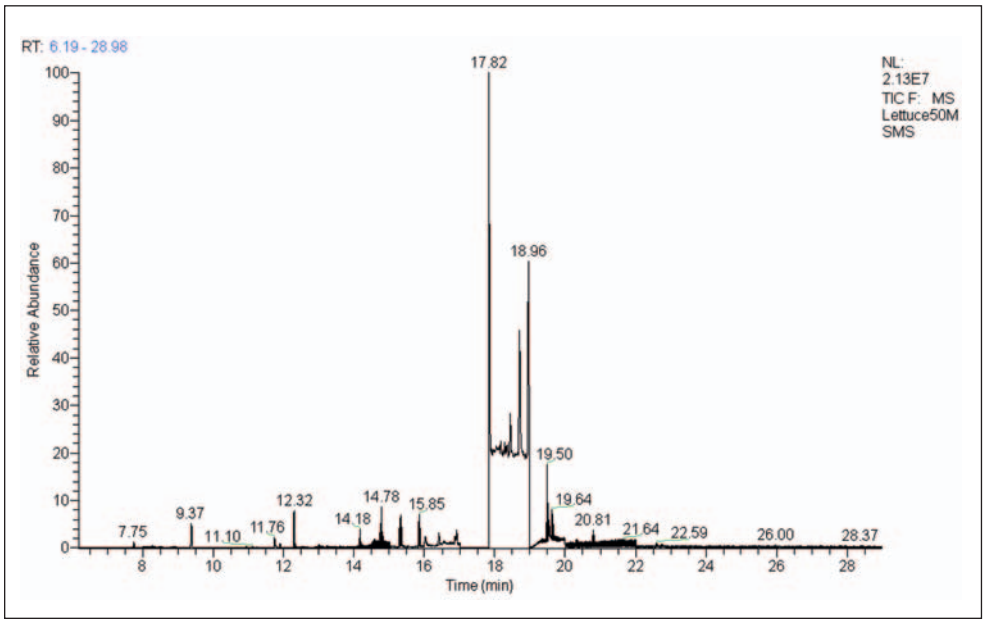

Figure 4: MS^n scan of 50 ng/g in lettuce matrix

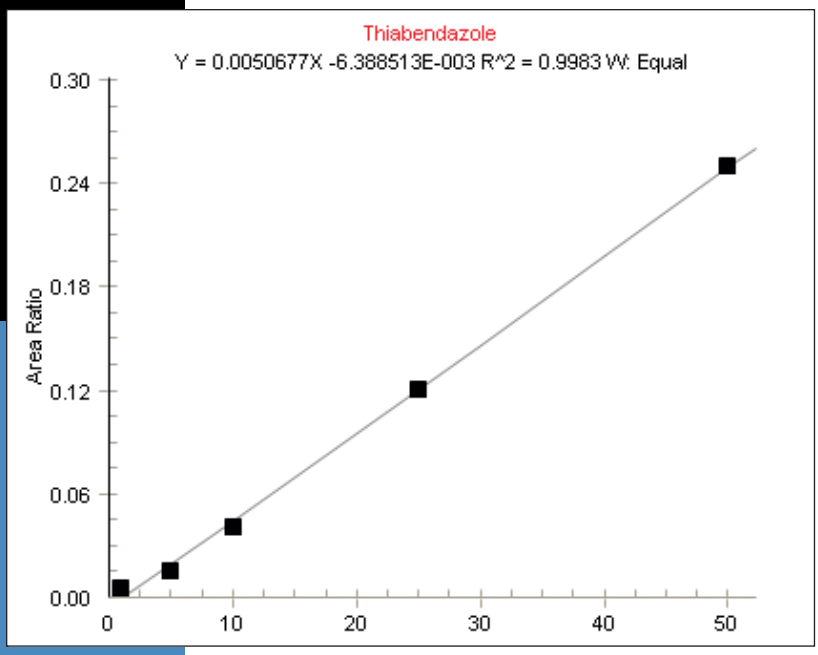

Figure 5: Calibration curve for thiabendazole in lettuce matrix

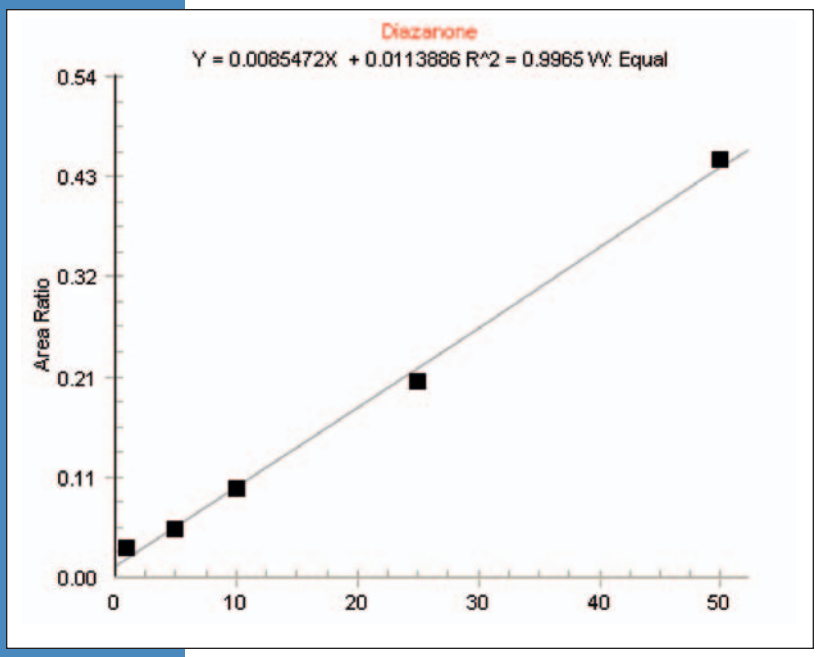

Figure 6: Calibration curve for diazinone in lettuce matrix

Results and Discussion

Linearity

A calibration curve was generated across five levels spiked into the lettuce matrix. Levels ranged from 1 ng/g to 50 ng/g, ensuring accurate quantitation at these lower concentrations. The linearity for most compounds was $R^2 > 0.99$. The precision of the internal standard and surrogate were 6 and 7% relative standard deviation (RSD) respectively. The results of the study are shown in Table 4. Figures 5 and 6 show two examples of calibration curves.

Component in Lettuce Matrix	Linearity (R^2)
mevinphos	0.9967
dimethoate	0.9976
gamma BHC	0.9957
diazanone	0.9965
chlorothalanil	0.9949
vinclozolin	0.9951
metalaxyl	0.9996
methiocarb	0.9957
dichlofluanid	0.995
cyprodinil	0.9958
thiabendazole	0.9983
folpet	0.9949
imazalil	0.9987
endosulfan sulfate	0.987
cis-permethrin	0.9966
trans-permethrin	0.9987
Average	**0.9961**

Table 4: Calibration Curve Results

Limits of Detection and Quantitation

The actual LOD and LOQ were determined by preparing matrix spikes at a concentration near the expected detection limit. A concentration of 5 ng/g was analyzed in eight matrix samples and the LOD and LOQ were calculated from these results by multiplying the standard deviations of the calculated amounts by 3 and 10 respectively. The recovery of the 5 ng/g standard ranged from 87-126% with an average of 105%, demonstrating the accuracy of this method at lower levels. The results are shown in Table 5.

Method Validation Results

The method validation calculations were performed using Thermo Scientific EnviroLab™ Forms data analysis and reporting software on four matrix samples spiked at a concentration of 50 ng/g. Samples had an average of 93% recovery with an average % RSD of 9.7. MVD results are shown in Table 6.

						WHO	Japan	EU	EU	US-EPA
Component	**Ave. Conc. (ng/g)**	**Std. Dev.**	**% RSD**	**LOD (ng/g)**	**LOQ (ng/g)**	**MRL[1] (ng/g)**	**MRL[2] (ng/g)**	**MRL[3] (ng/g)**	**LOD[3]**	**MRL[4] (ng/g)**
mevinphos	5.62	0.57	10.06	1.70	5.65		400	500		
dimethoate	5.01	1.27	25.24	3.79	12.65	2000	200	500	20	
gamma BHC	5.38	1.34	24.91	4.02	13.41		2000	10	10	3000
diazanone	5.18	1.23	23.66	3.68	12.26	500	100			700
chlorothalanil	5.73	0.91	15.91	2.73	9.11		1000			
vinclozolin	5.90	0.16	2.77	0.49	1.64	5000	5000			
metalaxyl	5.16	1.12	21.68	3.35	11.19	2000	2000	1000	50	5000
methiocarb	4.80	1.04	21.75	3.13	10.43	50	100			
dichlofluanid	5.38	0.96	17.89	2.88	9.62	10,000	10,000			
cyprodinil	6.29	0.30	4.78	0.90	3.01	10,000	1000			
thiabendazole	4.34	1.04	23.89	3.11	10.36		2000	50	50	
folpet	5.43	1.33	24.55	4.00	13.33	50,000	2000	2000		50,000
imazalil	4.55	0.72	15.70	2.14	7.15		20	20	20	
endosulfan sulfate	5.01	0.37	7.27	1.09	3.65	1000	1000	50	50	2000
cis-permethrin	4.70	0.94	19.91	2.81	9.36	2000*	3000*	50*	50*	20,000*
trans-permethrin	5.53	1.16	20.90	3.47	11.57					
Average	**5.25**		**17.56**	**2.71**	**9.02**					

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)
**Total Permethrins*

Table 5: Comparison of LODs and LOQs vs MRLs reported by various sources

Component in Lettuce Matrix	Average Concentration (ng/g)	Theoretical Concentration (ng/g)	% RSD	% Recovery
mevinphos	49.99	50	5.66	99.98
dimethoate	41.07	50	14.66	82.15
gamma BHC	47.40	50	4.16	94.80
diazanone	50.32	50	11.48	100.63
chlorothalanil	53.35	50	10.05	106.70
vinclozolin	47.40	50	12.00	94.81
metalaxyl	44.10	50	3.07	88.19
methiocarb	51.65	50	9.54	103.30
dichlofluanid	45.31	50	10.40	90.61
cyprodinil	44.43	50	9.30	88.85
thiabendazole	29.98	50	17.47	59.96
folpet	49.79	50	6.72	99.58
imazalil	38.72	50	15.89	77.43
endosulfan sulfate	53.72	50	6.25	107.44
cis-permethrin	51.50	50	10.54	103.01
trans-permethrin	47.26	50	7.92	94.51
Average	**46.62**			**93.25**

Table 6: Results of the method validation on the ITQ 700

Conclusions

The Thermo Scientific ITQ 700 GC-ion trap MS was thoroughly evaluated and showed excellent accuracy at low concentrations for a number of pesticide residues analyzed in iceberg lettuce. The injector demonstrated low endrin breakdown (< 10%) 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 $R^2 > 0.99$ for most compounds. The Method Validation Study generated an average % RSD of 9.7% for four replicate analyses at 50 ng/g and a calculated average LOD of 2.7 ng/g in iceberg lettuce was achieved based on 8 replicate analyses of 5 ng/g.

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
2. Rapid Method for the Determination of 180 Pesticide Residues in Foods by Gas Chromatography/Mass Spectrometry and Flame Photometric Detection, M. Okihashi, Journal Pesticide Science, 304 (4), (2005) 368–377
3. Commission Decision of August 12, 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, Official Journal of European Communities, 17.8.2002
4. MRLs for lettuce as listed at http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp

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