Technical Note: 10222

Analysis of Pesticide Residues in Lettuce Using a Modified QuEChERS Extraction Technique and Single Quadrupole GC/MS

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Key Words

- DSQ II GC/MS
- QuanLab Forms
- Food Safety
- QuEChERS
- Pesticide Residue Analysis

Introduction

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), and published recently as AOAC Method 2007.01.[1] The sample preparation is shortened 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 of pesticide residues in a lettuce matrix using gas chromatography/mass spectrometry (GC/MS) on the Thermo Scientific TRACE GC Ultra™ and Thermo Scientific DSQ™ II single quadrupole mass spectrometer. Thermo Scientific QuanLab Forms 2.5 software was used for data review and reporting. The MeCN extract is solvent exchanged to hexane/acetone for splitless injection with detection by electron ionization and selected ion monitoring (SIM).[2] A calibration curve was constructed in iceberg lettuce and then the precision and accuracy of the analytical method were tested by preparing matrix spikes at 5 ng/g and 50 ng/g.

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 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 various functional groups of most pesticides. A surge splitless injection was made into a Thermo Scientific TRACE™ TR-Pesticide capillary column (5% diphenyl/95% dimethyl polysiloxane column, (0.25 mm x 30 m, and a film thickness of 0.25 µm) with a guard column (0.25 mm x 5 m). The closed exit ion volume was used on the DSQ II. In order to test the implementation of the QuEChERS method, each facet of the method was evaluated to determine if any error may arise from slight modifications of the method. Since there are so many steps from sample preparation to actual detection on the MS, each portion of the method was studied separately. The following sections were evaluated:

- Sample Extraction and Clean Up
- Solvent Exchange
- Injection
- Separation
- Detection

Item Descriptions
TRACE TR-Pesticide (0.25 mm x 30 m, 0.25 µm with 5 m guard column)
5 mm ID liner, 105 mm long (pk of 5)
10 µL syringe
Septa (pk of 50)
Liner graphite seal (pk of 10)
Closed Exit Ion Volume and ion volume holder for DSQ II
Graphite ferrule 0.1-0.25 (pk of 10)
Ferrule, 0.4 mm ID 1/16 G/V
Blank vespel ferrule for MS Interface
2 mL amber glass vial, silanized glass, 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 (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

Sample Extraction and Clean Up

The QuEChERS sample prep procedure consists of the steps shown in Figure 1. There are three main parts: the extraction, clean up, and solvent exchange from acetonitrile (MeCN) to a solvent mixture of hexane and acetone (9:1). The solvent exchange provides a more amenable solvent for the splitless injection. Care must be taken to adequately homogenize the sample to the consistency of baby food or purée.

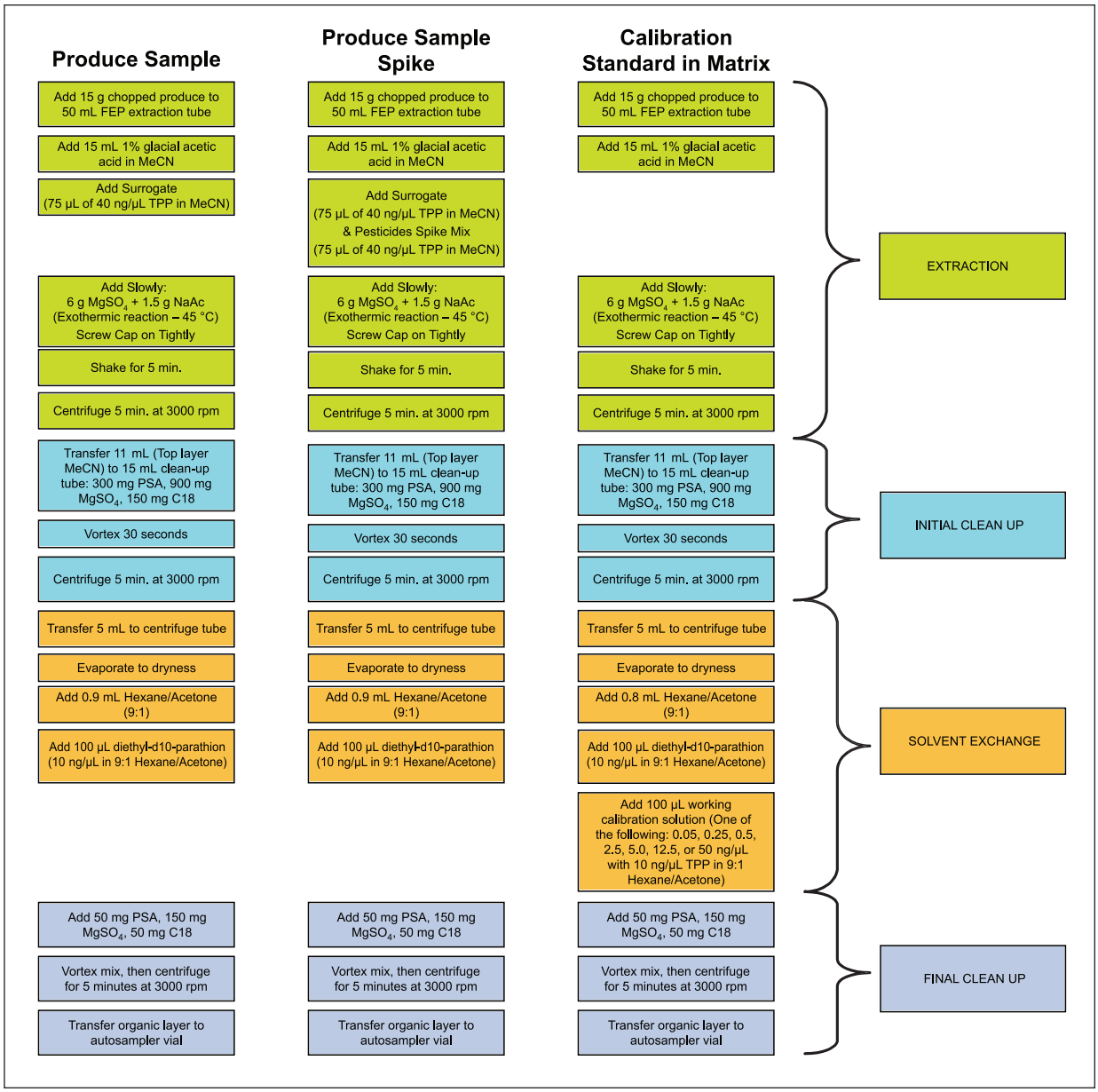

Figure 1: Flow Diagram of Modified QuEChERS Sample Prep

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 most vendors offer the pre-measured powder reagents in a separate capped extraction tube; these tubes should not be used, only the reagent in them. A change was implemented to add an empty 50 mL FEP extraction tube to the list of consumables for the sample preparation (Table 1). A well-homogenized 15 g sample of iceberg lettuce was weighed into this extraction tube. Then 15 mL of 1% glacial acetic acid:MeCN extraction solvent were poured into the tube on top of the sample. The surrogate was spiked into this MeCN layer along with the pesticide solution for the determination of the Method Validation Detection (MVD) and Limit of Detection (LOD). Then the tube was capped and vortex 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 then it was vortexed for 30 seconds until all of the powder reagents were mixed with the liquid layers. The tubes were placed on a mechanical shaker for 5 minutes. Then the tubes were centrifuged for 5 minutes at 3000 rpm. Next 11 mL of the top MeCN layer were removed and transferred to a 15 mL clean up tube. This tube was capped and vortexed for 30 seconds and then centrifuged for 5 minutes at 3000 rpm. Then 5 mL of the top layer were transferred into a clean test tube for solvent exchange.

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 not allow the tube to remain dry for more than a few minutes. 900 µL of hexane/acetone (9:1) were added and then 100 µL of the internal standard solution, d10-parathion, were spiked into the organic solution. The individual calibration levels were spiked in at this point for preparation of the calibration curve in matrix (Figure 1). The tube was capped and vortexed for 15 seconds. Then the 1 mL of extract was transferred to a 1 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 onto the GC/MS.

Injection

The injection must be optimized to inject the high and low molecular weight pesticides. The inlet temperature was set to 250 °C. This temperature was adequate to vaporize all of the pesticides studied. The 5 mm i.d. splitless liner with a volume of 1.6 mL was selected for the surged pressure injection. The inlet was set at an elevated pressure of 250 kPa for the 0.5 minute injection time. The vapor cloud is actually reduced for the 2 µL injection from 0.49 to 0.19 mL using this surge pressure injection mode. Then at an elevated injection flow rate of 4.7 mL/min, the liner is swept 1.5 times during the injection time. The target compounds move through the inlet so rapidly (10 seconds) that they do not have time to interact with the inside walls of the liner. The result is reduced breakdown of the more fragile pesticides. A Performance Solution was run at the beginning of each shift to test the endrin breakdown. This test proved that no maintenance was required. The results were < 5% endrin breakdown on a daily basis. This 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. Usually the liner is changed when the breakdown reaches > 20%. The injection port liner tested showed very good results, with minimal breakdown (Figure 2).

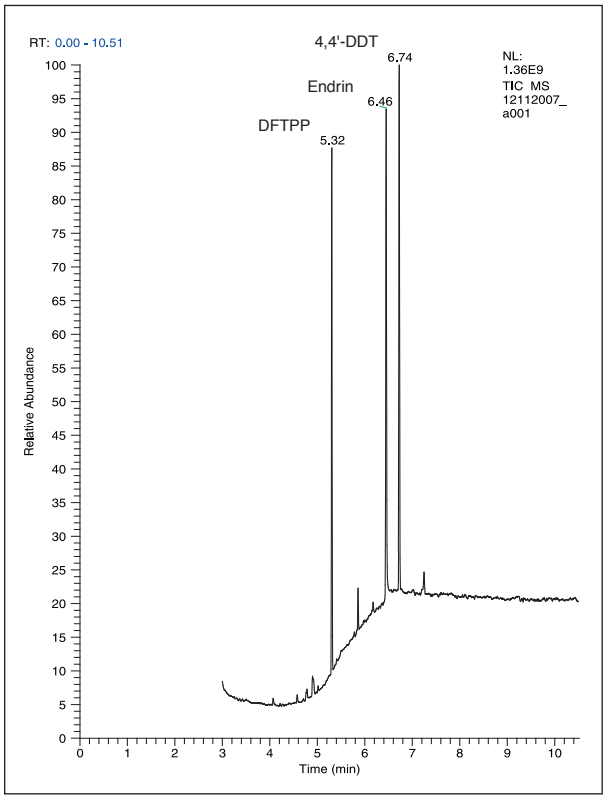

Figure 2: Total ion chromatogram of endrin breakdown QC test, demonstrating low system activity

AS 3000 Autosampler

Sample Volume	2 µL
Plunger Strokes	10
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
Pre-inject Solvent Wash Cycles	0
Sample Rinses	0
Post-inject Solvent	A
Post-inject Solvent Cycles	10

TRACE GC Ultra Gas Chromatograph

Column	TRACE TR-Pesticide 0.25 mm x 30 m, 0.25 µm with Integra-Guard Column (0.25 mm x 5 m)
Column Constant Flow	1 mL/min.
Oven Program	40°, 1.5 min., 25°/min.; 150°, 0.0 min., 7°/min., 225°, 0 min.; 25°/min., 290°, 10 min.
S/SL Temperature	250°
S/SL Mode	Splitless with Surge Pressure
Surge Pressure	250 kPa
Inject Time	0.5 min.
Split Flow	50 mL/min.
Transferline Temperature	290°

DSQ II Mass Spectrometer

Source Temperature	250°
Ion Volume	CEI
Emission Current	50 µA
Detector Gain	3 (1674V)
Lens 1	-25V
Lens 2	-5.4V
Lens 3	-25V
Prefilter Offset	-5.5
Electron Lens	15V
Electron Energy	-70V
Resolution Factors	Start Mass 1: 1.0, Ion Offset 1: 3.6, Res Factor 1: 1.89; Start Mass 2: 1050, Ion Offset 2: 3.6, Res Factor 2: 2.1
Tuning Factors	NA
Filament Delay Time	5.5 min.
End of Run Filament Off	25 min.
Tune	Autotune
Scan Parameters	(see Table 3)

Table 2: Selected instrument parameters for DSQ II, TRACE GC Ultra and Thermo Scientific AS 3000 autosampler

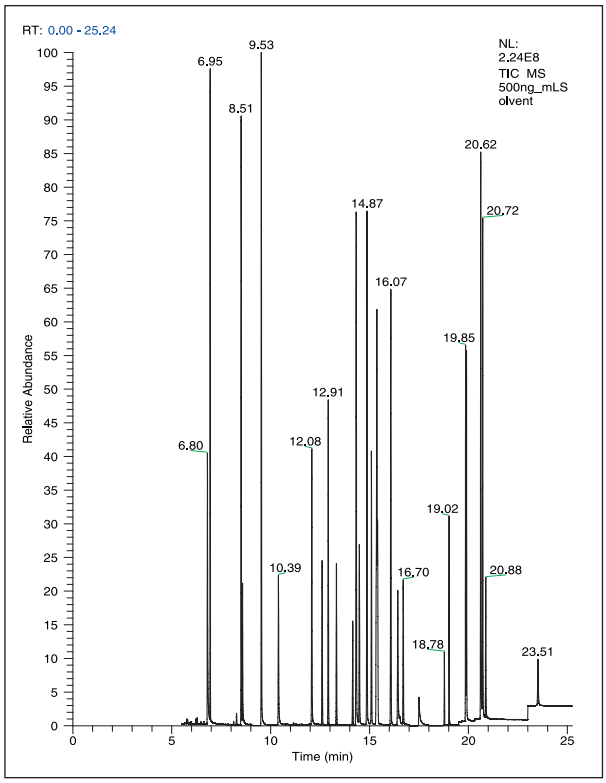

Figure 3: Pesticide Standard in Solvent at 500 ng/g (TIC of SIM)

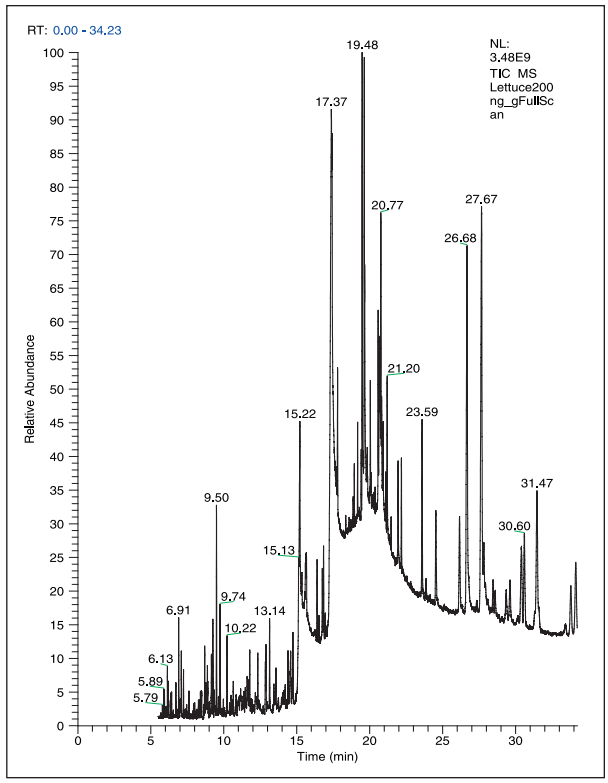

Figure 4: Iceberg Lettuce Matrix Spike at 200 ng/g in Full Scan

Separation

The separation was achieved by using a 5% diphenyl/ 95% dimethyl polysiloxane column, (0.25 mm x 30 m, and a film thickness of 0.25 µm) with a guard column (0.25 mm x 5 m). It is a non-polar phase and works quite well for heavily chlorinated pesticides. Some interactions within the stationary phase showed a loss of some pesticides at concentrations below 100 pg. These losses may be overcome by the addition of protectants.[5] The matrix-spiked calibration curve gave better linear fits than observed with the pesticide standards made in solvent only. This was due to the interaction of the matrix with the stationary phase, tying up active sites during the elution of the pesticide. The inlet was set at 250 °C and the MS source at 250 °C. The oven was programmed: 40 °C, 1.5 min., 15 °C/min., 150 °C; 7 °C/min., 225 °C; 25 °C/min., 290 °C, 15 min with a constant column flow rate of 1 mL/min.

The remaining instrument parameters are listed in Table 2. Separation of the pesticides studied was sufficient to set up the SIM ion windows for the analysis (Table 3). Deterioration of the peak shape that was observed for some pesticides when injected in solvent only was not observed when co-injected with matrix. A probable explanation is some activity in the flow path through the column. A total ion chromatogram (TIC) of the standard in solvent at 500 ng/mL is shown in Figure 3. An injection of the matrix extract in Full Scan was used to set the final hold temperature for the oven program (Figure 4). The filament was turned off after elution of the last pesticide in the final SIM method to help keep the mass spectrometer clean.

Compound	Retention Time	Segment #	Start Time (min.)	Quan Ion m/z	Quan Ion %	Qualifier Ions m/z	Qualifier Ions %	Qualifier Ions m/z	Qualifier Ions %	Width (amu)	Dwell Time (ms)
mevinphos	8.7	2	8.00	127	100	192	28	109	31	0.5	10
dimethoate	12.36			125	42	87	100	93	57	0.5	10
gamma BHC	12.86			219	49	181	100	217	40	0.5	10
diazinone	13.16	5	12.95	179	100	137	99	152	59	0.5	10
vinclozolin	14.42	6	14.00	285	41	178	99	212	100	0.5	10
metalaxyl	14.76			206	100	160	87	220	44	0.5	10
methiocarb	15.15	7	14.90	168	100	109	32	153	67	0.5	10
dichlofluanid	15.38			123	100	167	50	224	23	0.5	10
d10-parathion	15.61			301	40	99	100			0.5	10
cyprodinil	16.38	8	15.90	224	100	210	12	226	8	0.5	10
imazalil	17.72	9	17.20	215	100	173	86	217	63	0.5	100
endosulfan sulfate	18.95	10	18.50	272	100	274	75	229	71	0.5	50
TPP	19.17			326	100	325				0.5	50

Table 3: DSQ II SIM parameters for pesticides, surrogate and internal standard

Detection

The mass spectrometer scan speed was adjusted to accurately detect co-eluting pesticides. Ion ratios were monitored to prevent false positives from matrix interferences. The identification of the pesticides was performed by selected ion monitoring (SIM) by setting up discrete retention time windows and scanning events for prominent ions present in the pesticide (Table 3). Some overlays of ion ratio tests are shown in Figure 5. The closed exit ion volume was used on the DSQ II with an emission current of 50 µA.

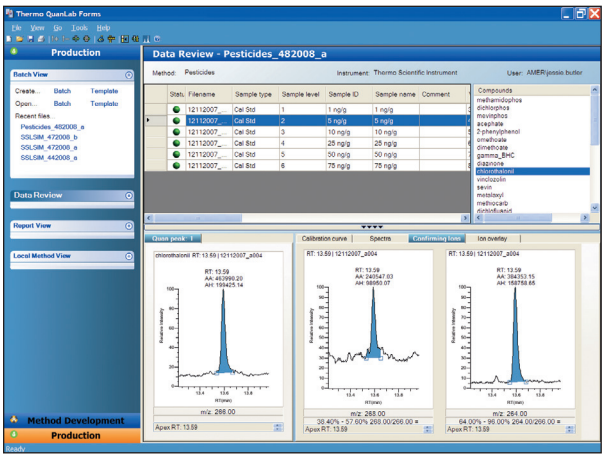

Figure 5: Overlay of Ion Ratios for chlorothalonil (5 ng/g)

Results and Discussion

A calibration curve was prepared in lettuce matrix and analyzed using Thermo Scientific QuanLab™ Forms reporting software, which measured the Pass/Fail of multiple Quality Control (QC) criteria specified in both AOAC Method 2007.01 and the European mass spectrometry identification criteria for SIM.[1,3] The internal standard used in the method was parathion-d10, and triphenylphosphate (TPP) served as the surrogate. Quantitation was based on linear least squares calibration with a correlation coefficient of $R^2 > 0.99$ for most pesticides. The average Limit of Detection (LOD) was 1.1 ng/g, well below most Method Regulatory Limits (MRLs) specified in CODEX.[4] The average Limit of Quantitation (LOQ) was 3.6 ng/g. The Method Validation study of four replicate analyses of 50 ng/g showed an average relative percent standard deviation of 10.5% and percent recoveries ranged from 68-102%, with an average percent recovery of 88%.

Linearity

The method specifies preparation of the calibration curve in matrix. They were prepared as shown in Figure 1. The average R^2 was 0.997. The results of the linearity study are shown in Table 4. Some typical calibration curve plots are shown for dimethoate and vinclozolin in Figures 6 and 7, respectively.

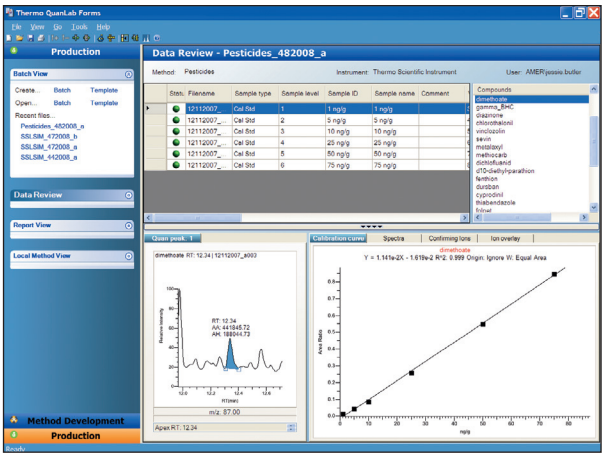

Figure 6: QuanLab Forms Data Review showing Dimethoate at 1 ng/g, with linearity from 1 ng/g to 75 ng/g

Figure 7: QuanLab Forms Data Review showing vinclozolin at 1 ng/g, with linearity from 1 ng/g to 75 ng/g

Component in Lettuce Matrix	Linearity (R^2)
mevinphos	0.9942
gamma BHC	0.9964
diazinone	0.9972
vinclozolin	0.9962
metalaxyl	0.9988
methiocarb	0.9956
dichlofluanid	0.9975
cyprodinil	0.9982
imazalil	0.9971
endosulfan sulfate	0.9972
Average	**0.9968**

Table 4: Pesticide calibration curve results, using linear least squares fit

MVDs

The replicate analyses of four matrix spikes at 50 ng/g provide information on the accuracy and precision of the method. In Table 5, the average calculated amount for the 50 ng/g spike in matrix was 44 ng/g. The percent recovery ranged from 68 to 102% with an average recovery of 88%. The precision of the MVD study was 10.5%RSD.

LOQs and LODs

The actual Limit of Quantitation (LOQ) was determined by preparing matrix spikes at a level 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 deviation by 3 and 10 respectively. The average calculated concentration of the spike was 5.4 ng/g. The average precision was 7.0%RSD and the average LOD was 1.1 ng/g with an average LOQ of 3.6 ng/g. The Method Regulatory Limits (MRLs) for the pesticides and the results of this study are shown in Table 6.

Component in Lettuce Matrix	Average Concentration (ng/g)	Theoretical Concentration (ng/g)	% RSD	% Recovery
mevinphos	42.5	50	11.0	85
gamma BHC	49.5	50	6.4	99
diazinone	51.1	50	6.1	102
vinclozolin	51.0	50	12.4	102
metalaxyl	44.9	50	4.8	90
methiocarb	38.9	50	14.8	78
dichlofluanid	41.4	50	13.4	83
cyprodinil	47.6	50	7.7	95
imazalil	34.1	50	12.0	68
endosulfan sulfate	39.3	50	16.3	79
Average	44.01		10.51	88.03

Table 5: Method Validation Results for pesticides in lettuce matrix

						WHO	Japan	EU	EU	US-EPA
Component	Ave. Conc. (ng/g)	Std. Dev.	% RSD	LOD	LOQ (ng/g)	MRL[1] (ng/g)	MRL[2] (ng/g)	MRL[3] (ng/g)	LOD_3	MRL[4] (ng/g)
mevinphos	4.21	0.61	14.5	1.83	6.10		400	500		
gamma BHC	5.26	0.368	7.0	1.10	3.68		2000	10	10	3000
diazinone	5.26	0.32	6.1	0.96	3.20	500	100			700
vinclozolin	5.97	0.205	3.4	0.62	2.05	5000	5000			
metalaxyl	5.12	0.24	4.7	0.72	2.40	2000	2000	1000	50	5000
methiocarb	5.47	0.21	3.8	0.63	2.10	50	100			
dichlofluanid	5.80	0.42	7.3	1.26	4.20	10,000	10,000			
cyprodinil	6.12	0.251	4.1	0.75	2.51	10,000	1000			
imazalil	4.70	0.574	12.2	1.72	5.74		20	20	20	
endosulfan sulfate	5.99	0.408	6.8	1.22	4.08	1000	1000	50	50	2000
Average	**5.39**		**6.99**	**1.08**	**3.61**					

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)
Values are listed in ng/g (ppb); converted to mg/kg (ppm) by dividing by 1000

Table 6: Comparison of limits of detection and quantitation to maximum residue limits (MRLs) from various agencies

Conclusion

AOAC Method 2007.01 was validated using the Thermo Scientific DSQ II operating in EI SIM. The DSQ II system is able to reliably meet detection limits and quality control requirements for determination of pesticide residues in lettuce using a modified QuEChERS sample preparation. The QuEChERS sample prep was modified to include a solvent exchange to hexane/acetone. The calibration curves for the pesticides studied met a linear least squares calibration with a correlation coefficient of $R^2 > 0.997$ for most compounds. The Method Validation Study generated an average %RSD of 10.5% for four replicate analyses at a 50 ng/g and a calculated average LOD of 1 ng/g in iceberg lettuce based on 8 replicate analyses of a 5ng/g with an average LOQ of 3.6 ng/g. The injector showed endrin breakdown at below 5% on a daily basis. The surged splitless injection with detection by three ion SIM met the criteria for the AOAC Method in iceberg lettuce matrix.

Reference

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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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
5. Combination of Analyte Protectants to Overcome Matrix Effects in Routine GC Analysis of Pesticide Residues in Food Matrixes, K. Mastovska, S. Lehotay, M. Anastassiades, Analytical Chemistry (77) (2005), 8129-8137

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