Implementation of QuEChERS Sample Prep in Routine Multi-residue Analysis of Pesticides in Fruits and Vegetables by GC/MS

Jessie Crockett Butler, David Steiniger, Eric Phillips

Thermo Fisher Scientific Inc. Austin, Texas, USA

Overview

Purpose: To test the implementation of the QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, **S**afe) sample prep procedures for GC/MS analysis by performing a large volume injection of extracts prepared in acetonitrile (MeCN). [1]

Methods: The extracts were prepared according to AOAC Method 2007.01 and injected via Programmed Temperature Vaporizing inlet (PTV) in the Large Volume Injection Mode (LVI) and detected using a Thermo Scientific DSQ™ II single quadrupole operated in EI selected ion monitoring (SIM).

Results: The linearity of a list of pesticides was evaluated in matrix. The Limit of Detection (LOD) and percent of recovery at a matrix spike level of 45 ng/g were determined. Precision was determined by running seven replicates of the matrix spiked standard.

Introduction

The analysis of pesticides in fruits and vegetables is dependent upon the efficiency of the extraction and accurate detection of low levels of pesticides in a high concentration of matrix background. The purpose of this study was to test extraction reproducibility in a spiked lettuce matrix and determine the sensitivity the Thermo Scientific DSQ II GC/MS system operated in SIM. The matrix chosen for the study was iceberg lettuce.

Methods

The validation of the method was divided into two phases: the sample preparation procedure and the instrument analysis method. The sample preparation process was evaluated to determine if minor modifications of the procedure would adversely affect the extraction efficiency. The validation of the instrument method was performed by working with the pesticide list in reagent solvent first and then in the matrix. The pesticide standard in reagent solvent was run to optimize the injection, separation and detection of the pesticides by setting up discreet SIM segments. Then the pesticide standard was spiked into the matrix and analyzed to determine the LOD and precision from the replicate runs of spiked matrix extracts.

Extracts were injected with a 10 µL syringe using the Thermo Scientific AS 3000 liquid autosampler on a Thermo Scientific TRACE GC Ultra™ configured with a PTV inlet with the LVI option (Figure 1). The DSQ II mass spectrometer was used with the closed exit ion volume (CEI). The mass spectrometer was set up for EI and SIM. The emission current was set to 50 µA at a Detector Gain of 3 using the Autotune Tune File. Each day a maintenance tune was performed to monitor the air/water spectrum and sensitivity of the mass spec.

Sample preparation was performed using Thermo Scientific HyperSep™ dispersive solid phase extraction (QuEChERS) products along with Fisher Scientific chemicals and solvents of the appropriate grade.

The following topics will be addressed in the Results section of this poster:

- Instrument Performance
- Sample Preparation
- Optimization of LVI
- Separation and Detection
- Method Validation: Linearity and LOD

Results: Instrument Performance

The instrument did not require cleaning during the study and the liner and column were not changed. At the beginning of each shift, a performance mix solution containing endrin and 4,4'-DDT was run to monitor the activity in the liner and column. The breakdown for endrin was calculated as shown below:

% breakdown = ((endrin aldehyde + endrin ketone)/ (endrin + endrin breakdown products)) X 100

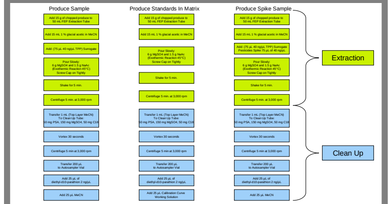
The daily endrin breakdown was < 10%. No leaks were observed and the response was stable.

FIGURE 1. DSQ II single quadrupole, TRACE GC Ultra, and AS 3000 autosampler

Results: Sample Preparation

The QuEChERS sample prep procedure consists of the steps shown in Figure 2. There are two main parts: the extraction and clean up. Since an LVI injection is made, the extraction solvent, acetonitrile (MeCN), will be injected and evaporated out of the split vent of the inlet to minimize the adverse effects of this polar solvent on the column. This also performs a 5X concentration of the extract. A solvent exchange would be required if the extracts will be injected into a standard splitless injector. This saves time in the sample preparation phase of the analysis.

FIGURE 2. Flow diagram of QuEChERS sample prep



Care must be taken to adequately homogenize the sample to the consistency of baby food or purée. The homogenized sample was then weighed into a 50 mL FEP extraction tube. Then the extraction solvent was added, along with any spiked standards. Finally the powdered reagents – magnesium sulfate and sodium acetate – were added slowly, due to the exothermic reaction resulting from the interaction with water in the sample. The tube was capped and put on a mechanical shaker for 5 minutes. After centrifuging, a 1 mL aliquot of the MeCN upper layer was transferred to a 1 mL clean-up tube. The clean-up tube was vortexed, centrifuged, and the extract was transferred to an autosampler vial for injection. Instrument parameters are shown in Table 1 below.

TABLE 1. Instrument Parameters

AS 3000 Autosampler:	
Sample Volume:	5 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 :	5
Sample Rinses:	0
Post-inject Solvent:	A
Post-inject Solvent Cycles:	10

DSQ II Mass Spectrometer:	
Source Temperature:	250°
Ion Volume:	CEI
Emission Current:	50 µA
Detector Gain:	3 (1674 V)
Lens 1:	-25V
Lens 2:	-5.4V
Lens 3:	-25V
Prefilter Offset:	15V
Electron Lens:	15V
Electron Energy:	-70 eV
Resolution Factors:	Start Mass: 1, 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:	N/A
Filament Delay Time:	6
End of Run Filament Off:	25 min.
Tune:	Autotune
Scan Parameters	See Table 2

TRACE GC Ultra:	
PTV Liner:	Siltek™ deactivated baffled liner
Column:	5% diphenyl 95% dimethyl polysiloxane 0.25 mm x 30 meter, 0.25 µm with guard Column (0.25 mm x 5 meter)
Column Constant Flow:	1 mL/min.
Oven Program:	50°, 0.1 min., 14.5°/sec.;110°, 0.5 min., 14.5°/sec., 275°, 1.0 min.; 275°, 20 min.
PTV Temperature:	70°, 1.5 min., 25°/min.;175°, 0.0 min., 5°/min., 225°, 0 min.; 25°/min., 290°, 6 min.
Mode:	LVI
Inject Time:	0.1 min.
Evaporation Time:	0.5 min.
Transfer Time	1.0 min
Clean Bake Out Time:	20 min.
Split Flow:	50 mL/min.
Solvent Valve:	128°
Transferline Temperature:	290°

Results: LVI Optimization

The parameters for the LVI injection are set based upon the volume of extract injected, the boiling point of the solvent, and the volatility of the target compounds and heavy matrices in the sample. For this application, the solvent was acetonitrile (MeCN) with a boiling point of 81.6 °C. A 5 µL injection was made. The final parameters are shown in Figure 3. The phases of an LVI injection are shown in the diagram in Figure 4, with a cross sectional view of the PTV with the baffled liner. The baffled liner provides a surface area for evaporation without the addition of glass wool, which is important because glass wool can cause loss of some very active pesticides.

FIGURE 3. Final PTV injection parameters

FIGURE 4. Diagram of LVI injection phases: injection, evaporation, transfer, and bakeout

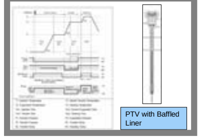
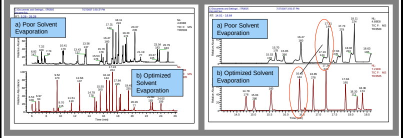


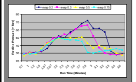
Figure 5 shows both an injection of a standard with too much solvent (a), creating skewed peak shapes and an optimized injection with Gaussian peak shapes with a narrow solvent plug (b).

FIGURE 5. Distortion of peak shape with poor evaporation parameters (a) and optimized parameters (b) of a standard with a 5 µL injection in acetonitrile



The width of the solvent plug may be measured by monitoring the rise in the vacuum of the mass spectrometer as the solvent enters the manifold. The solvent width is shown at various evaporation times (Figure 6). The optimum evaporation time selected was 0.5 minutes. At an evaporation time of 0.75 minutes, loss of the targets were observed. Figure 6 Also shows a step-by-step guide for setting up an LVI injection.

FIGURE 6. Solvent plug measured by monitoring the manifold pressure during elution of solvent at various evaporation times with a 5 µL injection of acetonitrile and general guidelines for setting LVI parameters



1) Is programmed injection required? Injection volume < 5 µL…NO
   > 5 µL Injection speed must be < than evaporation rate, requiring TriPlus Autosampler
2) Temperature of Injection: -10° below boiling point of solvent
3) Injection time = volume injection X speed of injection
4) Evaporation Temperature: > 10°C of boiling point of heaviest solvent if mixed solvents used.
5) Evaporation Time: dependent on volume injected and amount if residual solvent plug required
6) Transfer Temperature: > boiling point of heaviest target compound and < than late eluting matrix boiling point.
7) Transfer Time: 1 minute
8) Bake Temperature and Time: high enough to move matrix off of liner for duration of run

Results: Separation and Detection

Once the injection parameters have been optimized, the standard is run in full scan to determine the retention time for each pesticide. Retention time windows are made to set the segment breaks for the SIM ions for detection. The more ions in a segment window the shorter the dwell time must be set in order to detect each ion. The dwell time is determined by calculating how many scans will be taken across the width of a typical peak. Good precision comes from setting the appropriate dwell time to achieve the maximum number of scans across the peak. This is done by dividing the peak width (5 seconds) by the total scan time (0.33 seconds) and acquiring > 6 scans. Figure 7 shows the parameters in the Instrument Setup View for a segment with 9 SIM ions. The final SIM parameters for the method are listed in Table 2.

A full scan analysis of the sample matrix was performed to determine the final oven temperature for elution of the matrix. During the analysis of samples the filament is turned off after the last target compound elutes and the GC will continue to bake the column. This is a very important analysis to prevent the accumulation of matrix on the column and to minimize the contamination of the mass spec by the ionization of non-target compounds.

FIGURE 7. Scan parameters for SIM with view of scans across a peak

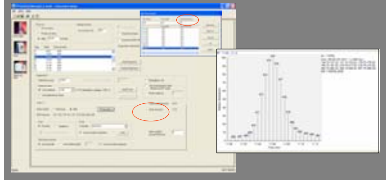


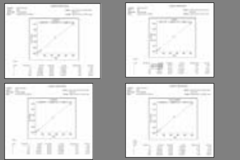
TABLE 2. DSQ II SIM ion parameters

				Quan Ion		Qualifier Ions					
seg. #	Start Tm.	Compound	Ret. Tm.	m/z	%	m/z	%	m/z	%	Width	Dwell Time (ms)
1	6.00	dichlorophos	6.49	109	100	185	30			0.5	50
2	8.00	2-phenylphenol	8.5	170	100	141	43	169	83	0.5	20
3	10.80	gamma BHC	11.05	219	65	181	100	217	50	0.5	20
		diazinone	11.19	179	95	137	60	152	70	0.5	20
		chlorothalonil	11.42	266	100	264	80	268	50	0.5	20
4	12.00	vinclozolin	12.55	285	50	198	95	212	100	0.5	20
		sevin	12.8	144	100	115	54	116	41	0.5	20
5	13.10	methiocarb	13.35	168	100	109	32	153	70	0.5	20
		dursban	13.77	197	100	199	96	258	42	0.5	20
		d10-parathion	13.89	301	100					0.5	20
		fenthion	13.89	278	100	125	50	109	40	0.5	20
6	14.40	cyprodinil	14.81	224	100	225	55			0.5	20
7	17.80	TPP	18.71	326	100					0.5	50
8	19.40	zolone EC	19.61	182	100	121	50	154	30	0.5	20
9	20.00	cis-permethrin	20.54	183	100	163	25	165	17	0.5	50
		trans-permethrin	20.65	183	100	163	30	165	21	0.5	50
		coumaphos	20.67	362	80	109	99	226	100	0.5	20
10	22.50	deltamethrin	23.56	181	100	172	40	253	40	0.5	100

Results: Validation Data – Linearity and LOD

A calibration curve was prepared in matrix as specified in the flow chart in Figure 1. The linearity was evaluated by applying a least square fit and determining the R^2 to be > 0.99. The results of the study are shown in Table 3 with graphic plots. The LOD was determined by preparing seven replicate spikes in lettuce at 45 ng/g.

TABLE 3. Linearity (5, 25, 50, 250, 500 ng/g) in iceberg lettuce matrix with selected calibration curves



Compound	R^2
dichlorphos	0.993
2-phenylphenol	0.998
dimethoate	0.997
gamma_BHC	0.997
diazinone	1.000
chlorothalonil	0.991
vinclozolin	1.000
sevin	0.995
methiocarb	0.996
dursban	0.999
fenthion	1.000
cyprodinil	0.999
zolone	1.000
cis-permethrin	0.998
trans-permethrin	0.998
coumaphos	0.999
deltamethrin	0.998

The comparison of the LODs to the MRLs as listed in Table 4, showed that the DSQ II provided more sensitivity than required to analyze at the MRL for the pesticides studied in lettuce. The average LOD was 8 ng/g. The average percent recovery of the 45 ng/g matrix spike was 95% with an average %RSD of 5%.

TABLE 4. LODs and MRLs for pesticides studied in iceberg lettuce (seven replicates at 45 ng/g)

Component	Ave. Conc.(ng/g)	Std Dev	%RSD	% Recovery	LOD(3X Std Dev)		WHO MRL[1]	Japan MRL2	EU MRL[3]	EU LOD[3]	US EPA MRL[4]
dichlorophos	31	3.68	12	70	11						
2-phenylphenol	48	1.36	3	106	4						
gamma_BHC	46	3.27	7	103	10			2,000	10	10	3,000
diazinone	50	1.13	2	111	3		500	100			700
vinclozolin	47	1.06	2	104	3		5,000	5,000			
chlorothalonil	36	4.37	12	80	13			1,000			
sevin	28	2.15	8	62	6						
methiocarb	31	1.77	6	69	5		50	100			
dursban	58	8.97	16	128	27						
d10-diethylparathion (ISTD)			2								
fenthion	49	3.71	8	109	11						
cyprodinil	41	0.89	2	92	3		10,000	1,000			
TPP(surrogate)		0.63	2								
zolone	37	1.03	3	82	3						
cis-permthrin	46	1.37	3	103	4		2,000*	3,000*	50*	50*	20,000
trans-permthrin	47	1.92	4	104	6						
coumaphos	38	1.38	4	85	4						
deltamethrin	23	2.56	11	52	8						
Ave	**41**		**5**	**95**	**8**						

[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

Conclusions

The DSQ II was evaluated for linearity and sensitivity for a group of pesticides in iceberg lettuce matrix. The QuEChERS sample preparation procedure specified in AOAC Method 2007.01 was used with direct injection of the acetonitrile extract using an temperature programmable inlet and a 5 µL large volume injection. The LVI and SIM parameters were optimized to provide accurate values for spiked pesticide standards in lettuce matrix. The linearity of all pesticides showed an R^2 fit of > 0.99. The average LOD was 8 ng/g with an average percent recovery of 95% and average precision of 5 %RSD.

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. *MRLs for lettuce as listed at http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp*

Siltek is a registered trademark of Restek Corporation. All other trademarks are property of Thermo Fisher Scientific Inc.

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC