Comparing LC and GC Triple Quadrupole MS for the Screening of 500 Pesticides in Matrix

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Overview

Purpose: The goal of this project is to compare the screening of more than 500 pesticides in matrix by LC and GC triple quadrupole, and determine the value of a comprehensive LC and GC screening approach.

Methods: The methodology included the vegetable extraction by QuEChERS followed by GC-MS/MS and LC-MS/MS analysis of over 500 pesticides in matrix.

Results: The majority of compounds could be detected to levels acceptable by EU standards by either GC/MS or LC/MS. All but eight pesticides could be determined to acceptable levels by the combined GC/LC methodology.

Introduction

Modern pesticide analysis is extremely challenging due to the diversity of compounds required to be reported, especially in the area of food safety control. Furthermore, the pressure to report large numbers of pesticides quickly makes it attractive to use large single injection methods. Triple quadrupole mass spectrometry has emerged as a primary technique for screening large target lists of pesticides due to its high sensitivity and selectivity against matrix. However, because of the chemical diversity of pesticides, LC or GC introduction alone may not be ideal, or even sufficient for a comprehensive analysis. Presented is a comparison of both LC and GC sample introduction techniques coupled to triple quadrupole mass spectrometer for the screening of more than 500 pesticides at ppb levels.

Methods

Sample Preparation

Pesticide standards were obtained from the U.S. Food and Drug Administration (FDA). In order to determine detection limits of such a wide range of pesticides, standards were prepared at multiple levels, enabling the selection of an appropriate level to determine the detection limit of each compound.

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Vegetable matrices were prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food[1]. The QuEChERS extracts were obtained from California Department of Food and Agriculture. For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used.

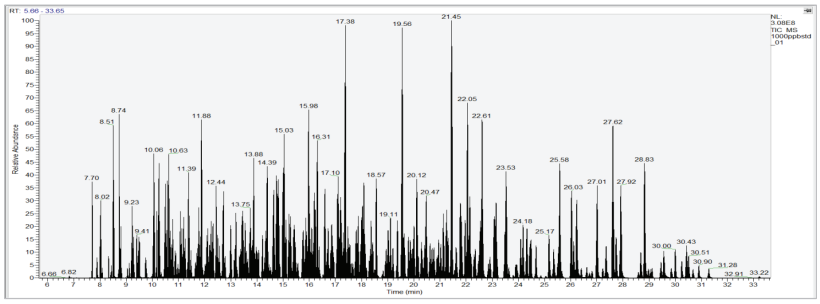
GC/MS Instrument Methodology

Gas Chromatograph Method Conditions

A method was developed for the Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and Thermo Scientific™ TSQ™ 8000 Mass Spectrometer. A Programmable Temperature Vaporization (PTV) injector was used on the TRACE 1310. The ability to program a temperature ramp with this injector was utilized so that thermally labile pesticides would be transferred to the analytical column at as low a temperature possible.

Similarly, the oven on the TRACE 1310 gas chromatograph was ramped, volatilizing pesticides on the column as their boiling points were reached. A slow ramp of 5 ºC/min was employed between an oven temperature of 180 ºC and 280 ºC, which is the range in which the majority of these pesticides are volatilized, to achieve optimal separation during this most dense part of the chromatogram. Figure 1 shows the total ion chromatogram resulting from the GC/MS method, and Figure 2 lists the GC method parameters.

FIGURE 1. GC/MS Total Ion Chromatogram.



The analytical column used was a Thermo Scientific™ TraceGOLD™ TG-5SILMS, with dimensions 30 m x 0.25 mm x 0.25 µm. The liner employed was a baffled, Siltek™ deactivated inlet liner.

FIGURE 2. Gas Chromatograph Parameters.

Injection Volume	
Injection Volume (µL):	1.0
Trace 1310 GC PTV Inlet	
PTV mode:	Splitless
Inlet (°C):	75
Split flow(ml/min):	50
Splitess time (min)	1
PTV inject:	75 °C , 0.1 min to transfer step
PTV transfer:	300 °C, 2.5 °C/sec for 3 min to clean step
PTV Clean:	330 °C, 14.5 °C/sec for 20 min
Carrier Flow He (mL/min):	1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Rate (°C/min)	25
Temperature 2 (°C):	90
Hold Time (min):	1.5
Rate (°C/min)	25
Temperature 3 (°C):	180
Hold Time (min):	0
Rate (°C/min)	5
Temperature 4 (°C):	280
Hold Time (min):	0
Rate (°C/min)	10
Temperature 5 (°C):	300
Hold Time (min):	5

GC-Triple Quadrupole Method Conditions

Transitions for all pesticides were taken from the Thermo Scientific™ TSQ 8000 Pesticide Analyzer. These transitions were originally developed with the use of AutoSRM software, which provided automated SRM development with collision energies optimized to ± 1 eV. Thermo Scientific TraceFinder™ software was used for acquisition and processing of the extracted samples. Selecting the appropriate compounds from the pesticide analyzer automatically populated the SRM acquisition list in the instrument method and the compound processing parameters in the Thermo Scientific™ TraceFinder™ software processing method. One ion per compound was used for quantitation and two additional ions were used for ion ratio confirmation. Figure 3 lists additional MS parameters used.

FIGURE 3. GC-Mass Spectrometer Parameters

Mass Spec Parameters	
Transfer line (°C):	**250**
Source temperature (°C):	**300**
Mode:	**SRM**
Ionization:	**EI, 70 eV**
Collision Gas:	**Argon**
Resolution:	**Q1 normal**

LC/MS Instrument Methodology

U-HPLC Method Conditions

Chromatographic analysis was performed using the Thermo Scientific™ Accela™ 1250 UHPLC system. The autosampler was an HTC-PAL™ Autosampler (CTC Analytics, Zwingen, Switzerland). The column used was a Thermo Scientific™ Hypersil™ GOLD aQ column (100 x 2.1 mm, 1.9 μm particle size). Displayed in Figure 4 is the total ion chromatogram. The UHPLC conditions are listed in Figure 5.

FIGURE 4. LC/MS Total Ion Chromatogram

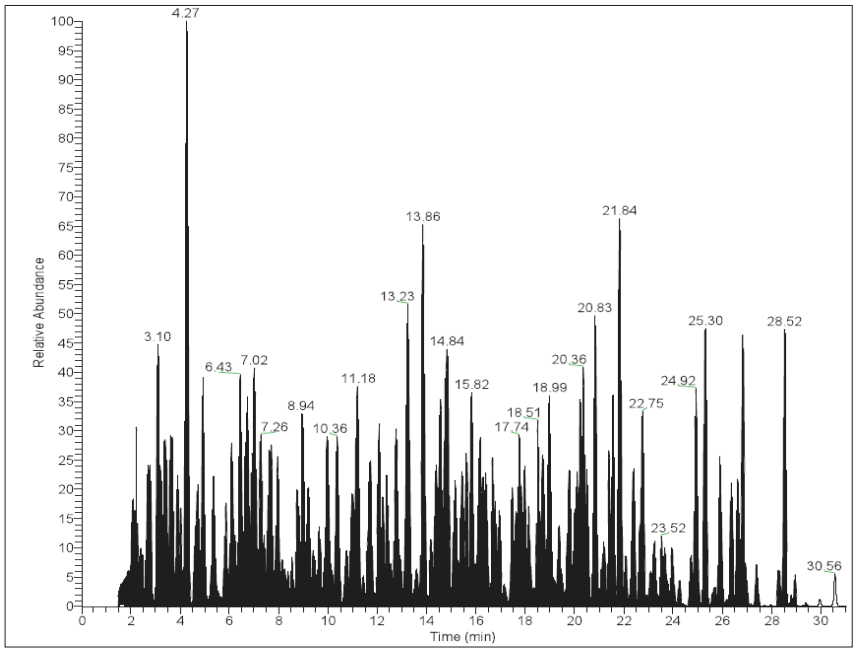


FIGURE 5. HPLC Parameters

HPLC Parameters			
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate		
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate		
Flow Rate:	300 µL/min		
Column Temperature:	40 °C		
Sample Injection Volume:	10 µL		
Gradient:	Gradient Time (min)	%A	%B
	0.00	98	2
	0.25	70	30
	35.00	0	100
	40.00	0	100
	40.01	98	2
	45.00	98	2

TSQ Quantum Access MAX LC-Triple Quadrupole Method Conditions

All samples were analyzed on the Thermo Scientific™ TSQ Quantum Access MAX™ triple stage quadrupole mass spectrometer with a heated electrospray ionization (HESI) source. To maximize the performance of the mass spectrometer, time-specific SRM windows were employed at the retention times of the target compounds. In addition, Quantitation-Enhanced Data-Dependent scanning, which delivers SRM-triggered MS/MS data, was used for structural confirmation. Alternating positive and negative polarity switching was utilized in the method. The MS conditions are listed in Figure 6 below.

FIGURE 6. LC-Mass Spectrometer Parameters.

Mass Spec Parameters	
Sheath Gas Flow Rate:	55 units
Aux Gas Flow Rate:	15 units
Spray Voltage:	3500 V
Capillary Temp:	280 °C
Heater Temp:	295 °C
Cycle Time:	0.2 s

Results and Discussion

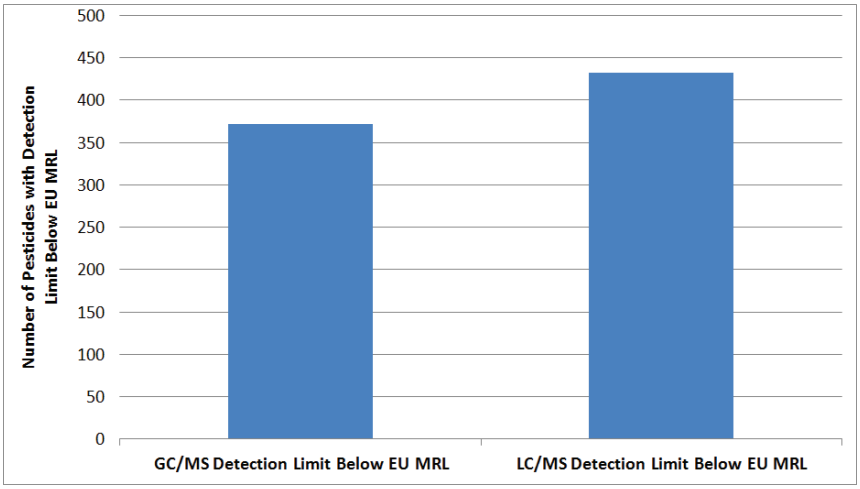
Determination of Method Detection Limit

For both GC/MS and LC/MS methods, spiked matrix samples were analyzed at several concentrations close to or below the European Union Method Reporting Limit (EU MRL). Each concentration level was injected several times and a statistical determination[2] of the method detection limit was calculated for comparison to the EU MRL for an onion matrix for each pesticide. When a required MRL was not available for the pesticide in onion, a 10 parts per billion MRL was used as stated in EU regulations.

Comparison of GC/MS to LC/MS

The majority of compounds were detected below EU MRLs by either the GC/MS or LC/MS method used (Figure 7). Out of the total 524 compounds analyzed, 372 pesticides had MDLs less than EU MRLs for the GC/MS methodology, compared with 432 pesticides with MDLs below the EU MRLs for the LC/MS methodology. Note that a10 µL injection was used in the LC/MS methodology compared with a 1 µL injection employed in the GC/MS methodology.

FIGURE 7. Number of compounds with method detection limits lower than EU MRLs for GC/MS and LC/MS methods

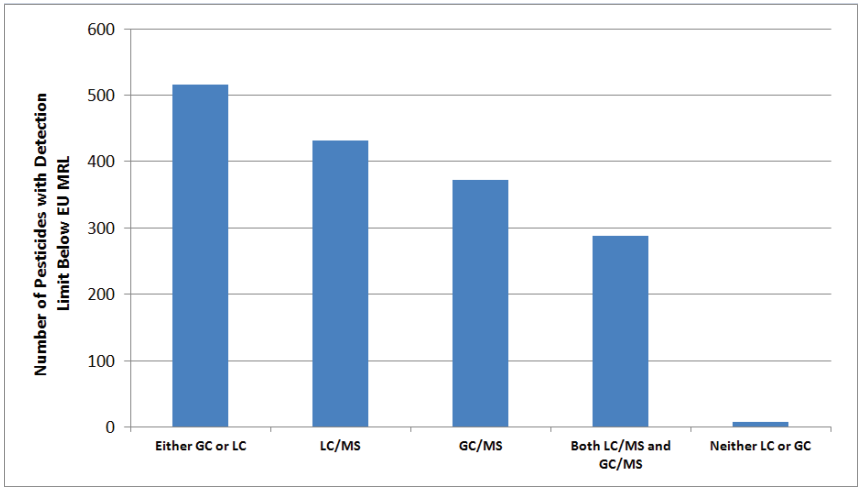


Benefits of Comprehensive GC/LC Methodology

By combining both GC and LC methodologies in a comprehensive screening methodology, 516 pesticides were detected below their MRLs for an onion matrix. This is 144 more than were detected below their MRLs for GC/MS methodology alone, and 84 more than by LC/MS alone. Only 8 pesticides had calculated detection limits for both GC/MS and LC/MS greater than their EU MRLs. On average, these 8 compounds" detection limits were four times their EU MRLs for the technique that gave them their lowest detection limit.

Furthermore, 288 compounds were able to be detected at concentrations below the EU MRL by both GC/MS and LC/MS methodology. This indicates that for a majority of these pesticides the two orthogonal techniques can be used together to increase confidence in the identification and quantitation. Figure 8 displayed below details these results.

FIGURE 8. Number of pesticides with detection limits below the EU MRL for GC/LC combined methodology compared with LC and GC methodology separately. Also displayed are numbers of pesticides detected below the MRL for both GC and LC methodology, and by neither methodology.



Conclusion

Methodology for both GC and LC/MS was developed and employed to analyze over 500 pesticides in a food matrix extracted with QuEChERS methodology. A summary of results, conclusions and possible future investigations for this project are as follow:

- 372 of 524 total pesticides were detected at levels under EU MRLs for onion samples by GC/MS
- 432 of 524 were detected at levels under EU MRLs for onion samples by LC/MS
- 516 of 524 were detected by either GC/MS, LC/MS, or by both GC/MS and LC/MS, demonstrating the power of combining these two techniques.
- For future work, a 10 µL large volume GC injection could be employed for the GC/MS methodology to better compare with the LC/MS methodology, and to try to lower the eight problematic pesticides detection limits under the EU MRL.
- Also, future work could explore techniques to selectively increase sensitivity for the eight problematic compounds, such as weighting SRM dwell time more heavily for these compounds, or decreasing resolution for these compounds, trading selectivity for sensitivity.

References

1. Steven J. Lehotay, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERs) Approach for Determining Pesticide Residues. Methods in Biotechnology, 2006, 19, 239-261.

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