# Broad Scope Pesticide Screening in Food Using Triple Quadrupole GC-MS

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# Overview

**Purpose:** To demonstrate two different ways to perform targeted and non-targeted screening of pesticides in one analytical run

**Methods:** Screening for 600 pesticides in selected reaction monitoring (SRM) mode or a smaller subset in selected reaction monitoring/ full scan (SRM/FS) mode

**Results:** Either method can be used to analyze targeted and non-targeted compounds with little loss of sensitivity

# Introduction

The increased accessibility of high selectivity GC-MS has enabled more generic sample preparation in pesticide testing, allowing consolidation of multiple analyte lists and matrices into one method. GC-MS/MS is well suited to multi-residue analysis in a diverse range of matrices. However, as the number of targeted compounds increases, the complexity of method optimization increases and analytical performance becomes compromised. Furthermore, there is a desire to look beyond targeted lists for other potentially harmful food contaminants. Presented here is the use of smart instrument control and data processing software applied to GC-MS/MS analysis of 600 pesticides in matrix to mitigate analytical performance degradation through MS duty cycle optimization. Also discussed is the combining of this optimized targeted quantitation with general unknown analysis through full scan/SRM.

# Method 1 – Screening For 600 Pesticides

### Sample Preparation

Lettuce was purchased from a local grocery store and was extracted with 1:1 ethyl acetate/cyclohexane following the QuEChERS method of extraction and clean-up, then 5 mL of solvent exchanged into 1 mL of hexane:acetone (9:1). The concentrated extract was spiked with various mixes of calibration standards.

### Gas Chromatography

The Thermo Scientific™ TRACE™ 1310 GC was equipped with both an SSL and PTV inlet. A 1 $\mu$L injection was performed on the PTV inlet. The liner was a Siltek™ deactivated baffled liner (Thermo Scientific part number 453T2120). Chromatographic separation was achieved by using a 5% diphenyl/95 % dimethyl polysiloxane column (30 m x 0.25 mm  0.25 $\mu$m). See Table 1 for the parameters for the PTV and oven.

**TABLE 1. PTV and Oven Parameters.**

| PTV | Mode | Temp | Split Flow | Splitless Time | Purge Flow |
|---|---|---|---|---|---|
| | Splitless | 75 | 50 | 2.00 | 5.00 |

| Flow Ramps | Rate (mL/min) | Flow (ml/min) | Hold (min) |
|---|---|---|---|
| | | 1.2 | 30 |
| | 2 | 3 | 7.2 |

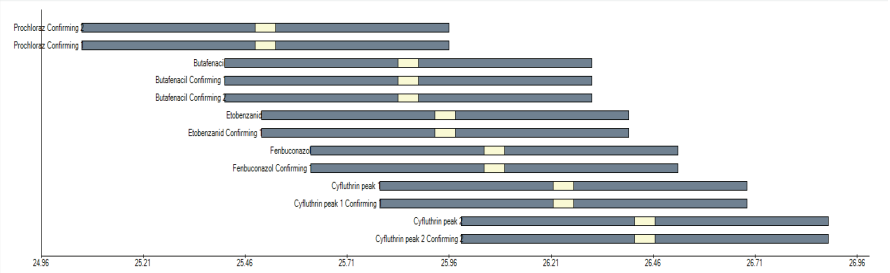
| Injection phases | Pressure (kPa) | Rate (°C/sec) | Temp (°C) | Time (min) | Flow (mL/min) |
|---|---|---|---|---|---|
| Injection | 70 | | | 0.1 | 50 |
| Transfer | 210 | 2.5 | 300 | 3.00 | |
| Cleaning | | 14.5 | 330 | 20 | 75 |

| Oven Program | Ramp | Rate (°C/min) | Temp (°C) | Hold Time (min) |
|---|---|---|---|---|
| | Initial | | 90 | 5 |
| | 1 | 25 | 180 | 0 |
| | 2 | 5 | 280 | 0 |
| | 3 | 10 | 300 | 5 |

### Mass Spectrometry

The targeted screening using SRM of 600 compounds was performed using the Thermo Scientific™ TSQ™ 8000 triple quadrupole MS. After retention times were determined in full scan, a timed-SRM method using selected reaction monitoring (SRM) was constructed to analyze all compounds in a single injection. Over 1,300 transitions were entered into the method from the TSQ 8000 Pesticide Analyzer Compound Database. This automatically populated both the processing and instrument method through the TSQ 8000 system Method Synch. The transfer line was set to 250 C, and the ion source was at 300 C. Figure 1 demonstrates timed-SRM (t-SRM) which allows for the analysis of the 600 pesticides and provides for good sensitivity.

**FIGURE 1. Small Section of Timed-SRM.**



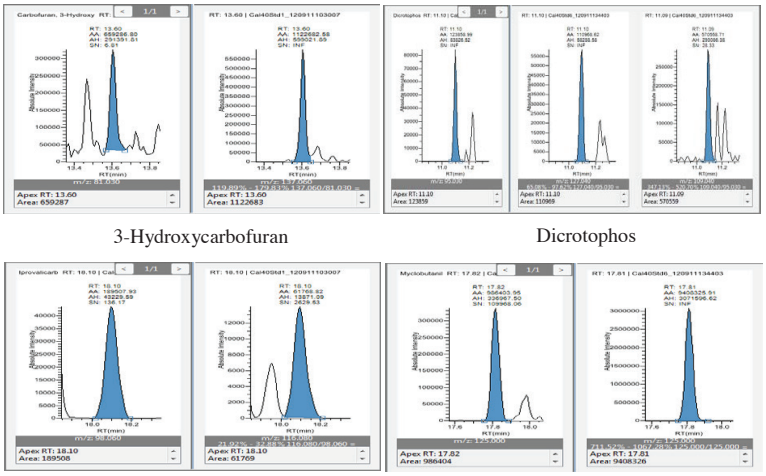

# Results

Quantitative performance was determined for 52 pesticides in lettuce matrix during the screening for all 600 pesticides. The linearity for all of the compounds was $R^2 > 0.98$. Curves were generated using Thermo Scientific™ TraceFinder™ software. Ten replicates of a 40 ppb matrix spike sample were also analyzed. To test screening capability, a few additional compounds were added to the 40 ppb spike which had not been part of the calibration, but could be identified through the use of this method. The average concentration and %RSD of the 40 ppb standard are given in Table 2. Figure 5 shows the quantitation ions and confirming ions of the compounds in the 40 ppb spiked sample that were not a part of the original calibration. This demonstrates the ability of the method and the instrument to identify targeted compounds in samples for which the instrument is not calibrated.

**TABLE 2. 40 ppb Standard Spiked into Lettuce Matrix.**

| Compound Name | Avg | %RSD | | Avg | %RSD |
|---|---|---|---|---|---|
| Acibenzolar-S-methyl | 32.1 | 8.8 | Flutolanil | 35.1 | 6.0 |
| Azinphos-methyl | 48.3 | 4.4 | Fuberidazol | 45.5 | 9.8 |
| Azoxystrobin | 39.5 | 2.3 | Furalaxyl | 62.4 | 4.4 |
| Benalaxyl | 43.8 | 6.3 | Imazalil | 45.6 | 3.5 |
| Bendiocarb | 50.7 | 3.9 | Indoxacarb | 47.2 | 9.2 |
| Bitertanol | 48.4 | 7.1 | Isoprocarb | 43.9 | 2.3 |
| Boscalid (Nicobifen) | 44.0 | 3.2 | Mefenacet | 47.1 | 2.9 |
| Buprofezin | 39.6 | 5.5 | Metalaxyl | 38.8 | 8.3 |
| Carbaryl | 56.1 | 2.3 | Methiocarb | 58.7 | 4.0 |
| Carbofuran | 45.1 | 11.8 | Mevinphos | 46.2 | 6.0 |
| Carboxin | 44.6 | 4.2 | Oxadixyl | 41.4 | 4.6 |
| Carfentrazon-ethyl | 39.1 | 5.4 | Piperonyl butoxide | 42.6 | 2.0 |
| Clethodim | 30.6 | 15.4 | Pirimicarb | 26.6 | 16.5 |
| Cyprodinil | 42.5 | 2.9 | Propargite | 55.9 | 6.5 |
| Diethofencarb | 41.2 | 6.7 | Propham | 40.2 | 1.7 |
| Difenoconazole peak 1 | 53.7 | 3.0 | Propiconazole peak 1 | 43.7 | 18.5 |
| Difenoconazole peak 2 | 45.5 | 3.6 | Propiconazole peak 2 | 49.3 | 6.0 |
| Dimethomorph-1 | 52.8 | 7.1 | Propoxur | 46.9 | 2.1 |
| Dimethomorph-2 | 49.7 | 3.2 | Pyridaben | 39.0 | 1.4 |
| Ethofumesate | 40.9 | 4.3 | Pyrimethanil | 37.5 | 15.3 |
| Fenamidone | 49.8 | 5.0 | Spiromesifen | 62.8 | 6.0 |
| Fenbuconazol | 40.7 | 1.2 | Spiroxamine | 52.3 | 7.0 |
| Fenoxycarb | 44.4 | 3.0 | Thiabendazole | 49.6 | 9.9 |
| Flonicamid | 44.7 | 6.1 | Triazophos | 46.7 | 4.3 |
| Fludioxonil | 45.2 | 5.7 | Triflumizole | 48.5 | 14.3 |
| Flusilazole | 44.8 | 6.1 | Zoxamide | 58.6 | 4.3 |

**FIGURE 2. Pesticides Identified by Ion Ratio Not in the Targeted Calibration Curve. First Peak is the Quan Peak, and the Others are for Confirmation.**

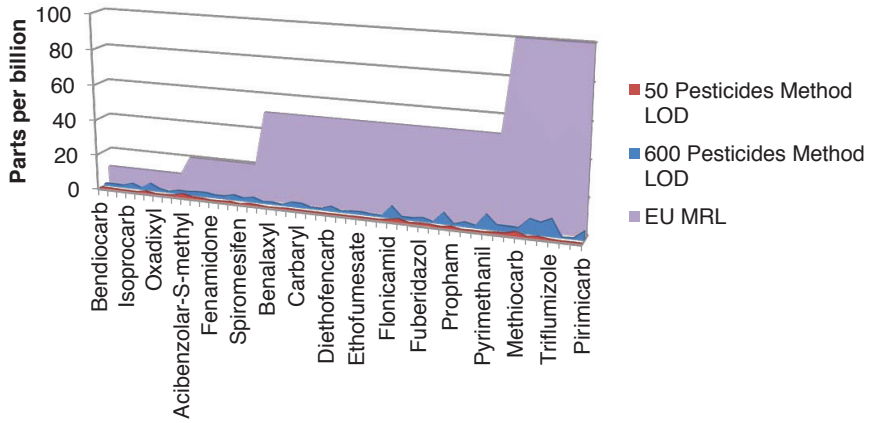


3-Hydroxycarbofuran Dicrotophos

Improvalicarb Myclobutanil

A second method was generated that targeted only the 52 compounds and contained only 104 transitions. Ten replicates of a 5 ppb and 10 ppb standard were analyzed to determine the MDLs for the two instrument methods, one with 1300+ transitions, and the other containing only 104 transitions. The results of compounds with MRLs for lettuce are shown in Figure 3. Although lower detection limits result from longer dwell times in the method with 104 transitions, the screening method that scans for 600 compounds is still capable of reaching the limits in lettuce set by the EU for the compounds requiring a targeted analysis in our list.

**FIGURE 3. Comparison of MDLs: 52 Compounds vs. 600 Compounds.**




# Method 2 – Alternating SRM/FS

### Sample Preparation and Gas Chromatography

The sample preparation and GC parameters remained the same as in the first study.

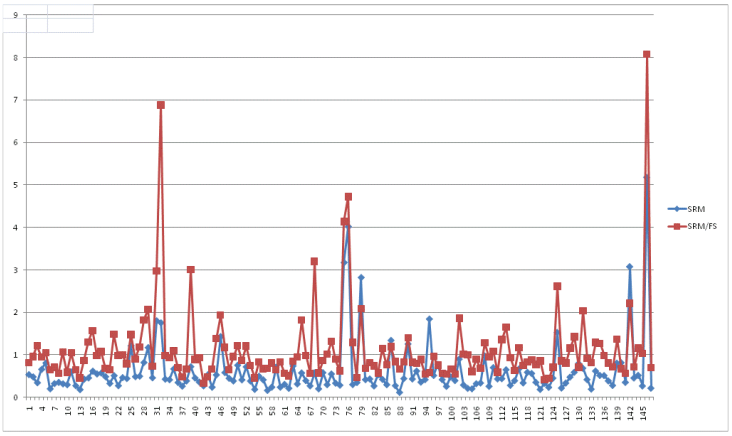
### Mass Spectrometry

The scanning of 147 compounds was performed using the TSQ 8000 triple quadrupole MS. After retention times were determined in full scan, a timed-SRM method using selected reaction monitoring (SRM) was constructed to analyze all 147 compounds in a single injection. A second method was constructed, adding full scan to the analysis.

# Results

A sample of fruit drink was extracted using the QuEChERS method of extraction and cleanup. The extract was concentrated 5x, then 147 pesticides were spiked into the extract to produce calibration curves from 1 ppb to 200 ppb. The calibration curves were constructed using TraceFinder software for both methods, SRM and alternating SRM/full scan for 147 pesticides. The linearity for most of the compounds was $R^2 >$ 0.98 for both methods of analysis. Ten replicates of a 1 ppb and 10 ppb standard in fruit juice extract were analyzed to determine the MDLs for the two instrument methods, SRM only and alternating SRM/full scan A comparison of the MDLs of both methods are shown in Figure 4. MDLs are slightly higher with the full scan added to the instrument method, but very comparable.

**FIGURE 4. Comparison of MDLs from SRM vs. SRM/FS analysis (ppb).**




Fruit drink was spiked at 100 ppb and analyzed using the SRM/FS instrument mode. This extract was also spiked with two phthalates at a 1 ppm level. The full scan chromatogram shows several peaks above the 100 ppb pesticide spike. Peaks are at retention times of 9.29, 9.73, 10.39, 10.91, and a very large saturated peak at 31.00 minutes. A close-up view of the first four compounds is shown in Figure 5. Figure 6 displays the NIST library matches for those non-targeted compounds.

**FIGURE 5. Close-up View of Four Unknown Peaks in 100 ppb Spiked Fruit Drink.**

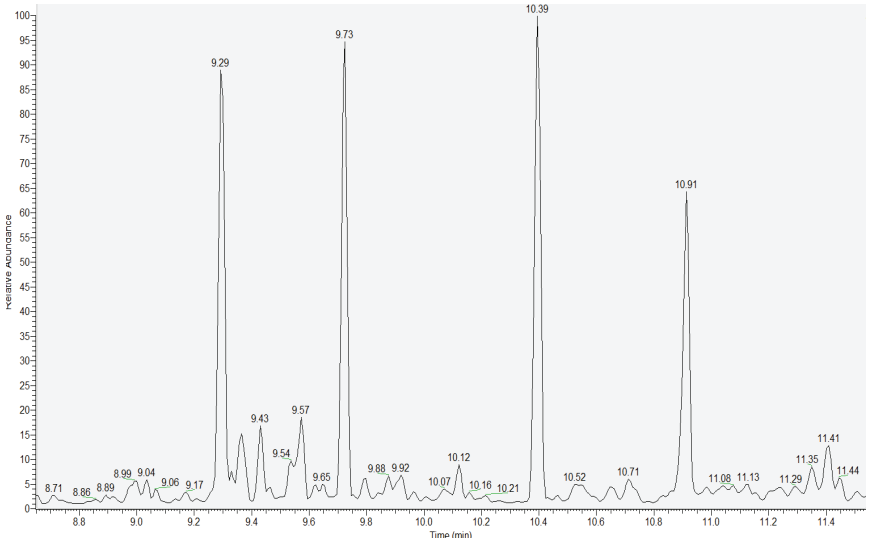
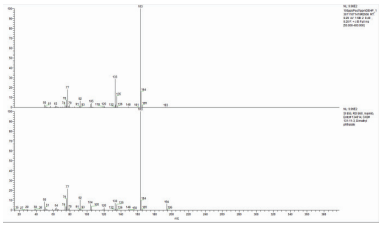
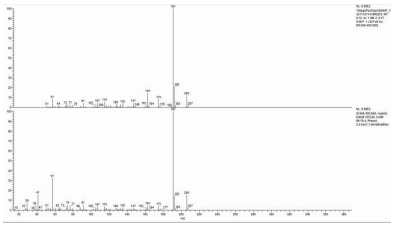


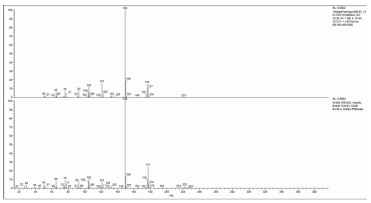
**FIGURE 6. NIST Library Match for 4 Unknown Peaks.**



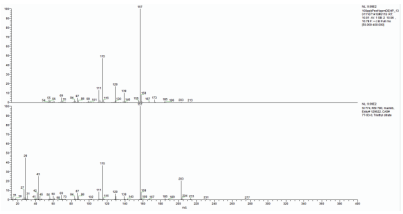
Dimethyl phthalate at 9.29 min



Phenol, 2,4-bis (1,1-dimethylethyl) at 9.72 min
used as a UV stabilizer in plastics



Diethyl phthalate at10.39 min



Triethyl citrate at 0.91 min
used as a plasticizer and food additive

# Conclusion

Two different ways of analyzing targeted and non-targeted compounds have been demonstrated using the TSQ 8000 MS paired with the TRACE 1310 GC. Method 1 utilized the high SRM scan rate of the TSQ 8000 to scan for 600 pesticides in one analytical run without sacrificing sensitivity. Without having to calibrate all 600 pesticides, an analyst can still identify additional pesticides that may appear in the sample. Method 2 utilizes the ability of the TSQ 8000 to generate high quality library searchable full scan spectra at high scan speeds by operating the instrument in SRM/FS mode. This was done by selecting a number of target compounds for low level SRM analysis, while using full scan to identify unknowns of any classification, such as leachates from packaging, or nutritional compounds and preservatives added to food products.

Listed below is a summary of the two methods.

**Screening for 600 Pesticides**

- Screening for 600 pesticides without sacrificing sensitivity due to the high scan speed of the TSQ 8000
- 52 compounds calibrated with $R^2 > 0.98$
- Ability to identify pesticides not in the calibration through ion ratios
- Customizable compound list using AutoSRM feature to optimize new compounds

**Alternating SRM/FS**

- Target large number of compounds while collecting full scan data
- Quantitate targeted compounds while looking for non-targeted compounds
- Unknown identification of non-targeted compounds using the NIST library
- Calibration curves for most pesticides were $R^2 > 0.98$
- Comparable MDLs with or without full scan data collection
- Can be used for identifying contamination from packaging, nutritional components, or preservatives added to food products
- Customizable compound list using AutoSRM to optimize new compounds

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