

# Fast Screening for Pesticides in Fruits and Vegetables by Gas Chromatography—Time-of-Flight Mass Spectrometry

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**Key Words:** GC-TOFMS, Pesticides, Food Analysis

## 1. Introduction

Identifying and quantifying pesticides in fruits and vegetables is important for public health and safety reasons. As sample loads increase, analysts look for ways to enhance the speed of sample preparation and analysis while maintaining high data quality.

The United States Department of Agriculture has implemented the Pesticide Data Program (PDP) to provide data on pesticide residues in food, through an Annual Summary. These data are subsequently used by the Environmental Protection Agency (EPA) to conduct dietary risk assessments for adults, and especially children and infants. One of the most important outcomes of the PDP Annual Summary is the information on pesticides likely to be detected in certain commodities, their concentration ranges, and whether or not EPA tolerances have been violated.

This application note describes the results from using a fast sample preparation method and fast GC—Time-of-Flight Mass Spectrometry (TOFMS) to measure pesticides in fruits and vegetables. Fruit and vegetable extracts were prepared and spiked with pesticides to determine the ability of the GC-TOFMS to identify and quantify pesticides in the presence of sometimes overwhelming matrix components. The commodities and pesticides were chosen based on their appearance in the PDP Annual Summary for Calendar Year 2000. The spike levels were always lower than the EPA Tolerances listed in the Annual Summary (where available), most of the time by an order-of-magnitude.

## 2. Experimental Conditions

**Standards:** Custom Pesticide Mixes (Restek)

**Gas Chromatography:** Agilent 6890

**Column:**

30 m x 0.25 mm x 0.25  $\mu$ m Rtx-PCB (Restek)

**Carrier:**

Helium at 1 ml/minute, constant flow

**Injection:**

1  $\mu$ l splitless at 250°C, 60 second valve

**Oven Program:**

60°C (1 minute), 30°/minute to 120°,

15°/minute to 360° (1 minute)

**Total Run Time:** 20 minutes

**Mass Spectrometry:** LECO Pegasus III GC-TOFMS

**Ionization:** Electron ionization at 70eV

**Source Temperature:** 225°C

**Stored Mass Range:** 45 to 550 u

**Acquisition Rate:** 10 spectra/second

**Data Processing:**

LECO ChromaTOF software with Automated Peak Find and Deconvolution

**Extraction of Fruits and Vegetables**

Fruits and vegetables, chosen for this work based on their appearance in the PDP Annual Summary 2000, were obtained from a local grocery store. The fruits were red seedless grapes from California, and California navel oranges (Figure 1). Vegetables included red leaf lettuce, California bunch carrots, and cucumber (Figure 2).



Figure 1. Grapes and oranges picked for pesticide screening.



Figure 2. Lettuce, carrots, and cucumber picked for pesticide screening.

Prior to extraction, each commodity was rinsed with organic-free water. Grapes were separated from stems, and the stems were discarded. The rind was removed from oranges and discarded. Only the root was used for carrot extraction. Leaves were stripped from the lettuce to use for sample preparation. If necessary, each commodity was chopped into smaller pieces with a knife

prior to homogenization in a glass beaker with a handheld bamix de luxe homogenizer.

A modified QuEChERS method (Lehotay, et al.) was used to extract homogenized commodity. 20 ml of acetonitrile (containing a surrogate mix of 4-chloro-3-nitrobenzotrifluoride and 4,4'-dibromobiphenyl) was shaken with 20 g of commodity in a glass jar for 1 minute. 8 g of  $MgSO_4$  and 2 g NaCl were added to the jar contents, followed by another 1 minute shake. After a brief settling period, 5 ml of extract was removed to a small PTFE centrifuge tube for dispersive solid phase cleanup. 125 mg aminopropyl sorbent and 750 mg  $MgSO_4$  were added to the tube and the tube was shaken for 30 seconds and then centrifuged for 1 minute. The clear extract was then removed to another vial for GC-TOFMS analysis.

### 3. Results

#### Analysis of Standards

Mixed standards representing a total of over 150 pesticide compounds at several concentration levels were analyzed by GC-TOFMS to produce the calibration curves necessary for quantification. A chromatogram of a standard representing all of the pesticides is shown in Figure 3. The peak labeled as Methomyl is likely a degradation compound of the parent carbamate pesticide. To conserve space, a listing of the pesticides will not be presented here, but the chemical classes include: carbamates, organophosphorus, organochlorine and other organohalogens, organonitrogens and triazines, pyrethroids, and others. Herbicides, fungicides, insecticides, and degradation compounds are represented in the standard.

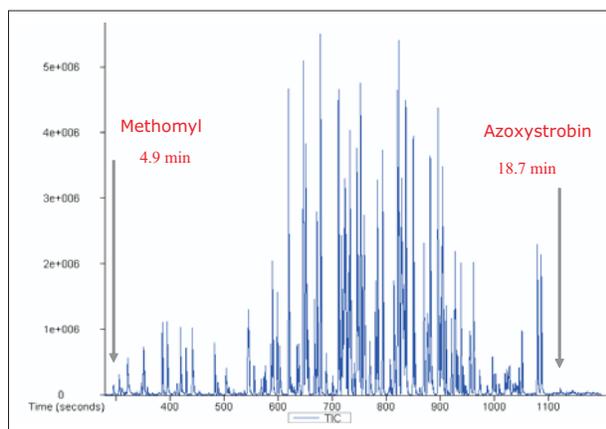


Figure 3. Chromatogram of pesticide standard containing over 150 compounds.

In a chromatogram as complex as Figure 3 there will be many coelutions, but as long as they are not exact coelutions, the Automated Peak Find and Spectral Deconvolution algorithms of ChromaTOF allow production of good quality spectra. This is demonstrated in Figures 4 through 9.

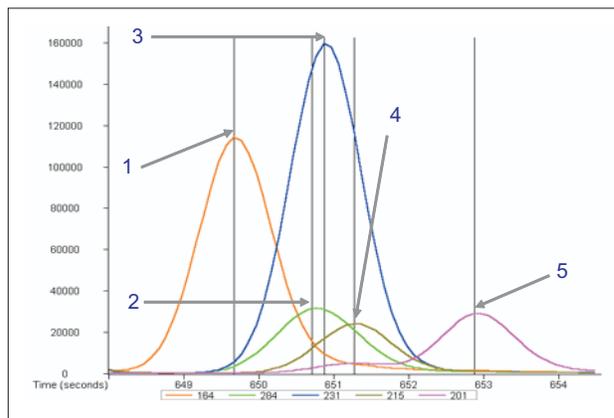


Figure 4. Chromatogram showing automatic peak find (peak markers are vertical lines) for five pesticides eluting in a five second window. Peaks 2 and 3 are separated by approximately 150 ms. Deconvoluted mass spectra are shown in Figures 5 through 9. Peak identities: (1) Carbofuran; (2) Hexachlorobenzene; (3) Terbufos; (4) Atrazine; (5) Simazine.

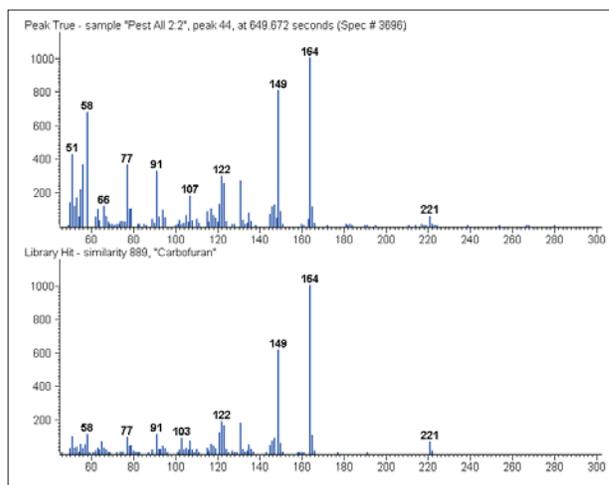


Figure 5. Deconvoluted mass spectrum for Peak 1 (Carbofuran) in Figure 4 and NIST library mass spectrum.

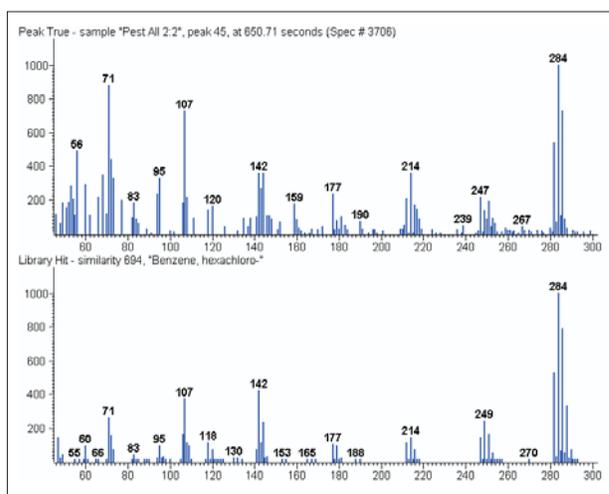


Figure 6. Deconvoluted mass spectrum for Peak 2 (Hexachlorobenzene) in Figure 4 and NIST library mass spectrum.

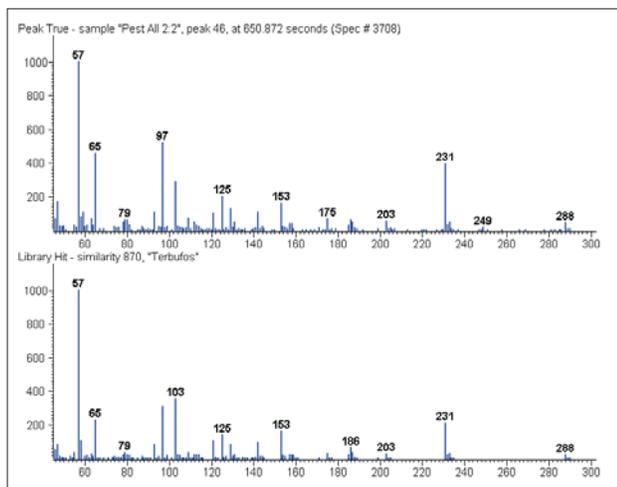


Figure 7. Deconvoluted mass spectrum for Peak 3 (Terbufos) in Figure 4 and NIST library mass spectrum.

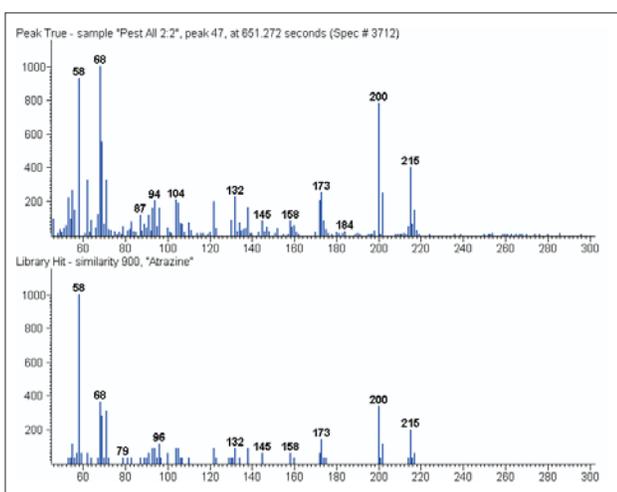


Figure 8. Deconvoluted mass spectrum for Peak 4 (Atrazine) in Figure 4 and NIST library mass spectrum.

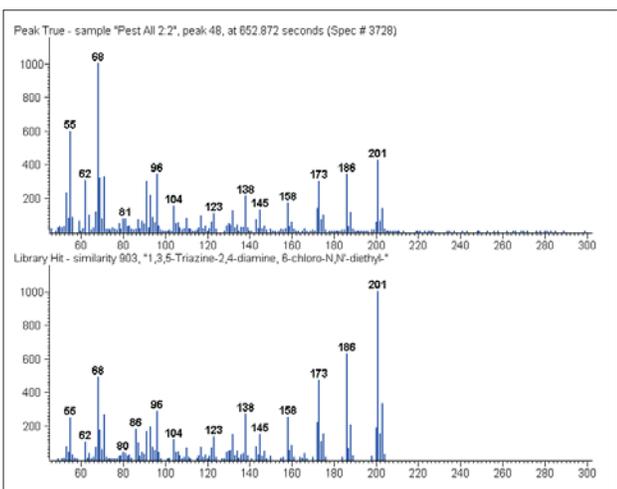


Figure 9. Deconvoluted mass spectrum for Peak 5 (Simazine) in Figure 4 and NIST library mass spectrum.

### Calibration and Analysis of Extracts

Before analysis, each extract was spiked with an internal standard (pentachloronitrobenzene) and quantified for pesticides based on calibration curves that ranged from very low pg/ $\mu$ l to low ng/ $\mu$ l in concentration. Example calibration curves are shown in Figure 10.

Calibration and Quantification with TOFMS are similar to what would be performed in a selected ion recording experiment with quadrupole or magnetic mass spectrometers—i.e. one or a few ions are used for quantification masses in calibration curve preparation (e.g. Trifluralin calibration is based on summed ions 264 and 306). There is one huge difference though... TOFMS provides a full mass spectrum of the pesticide even at low pg levels. The mass spectrum from a Standard is used as a Reference, to reverse match against sample spectra when doing target pesticide work in a fruit or vegetable matrix.

It should be noted that the standards for all of the quantification were in solvent only, and not in any matrix. Matrix-enhanced response, which is typical for certain active pesticides, is acknowledged, but to calibrate for all chosen matrices using standards in matrix is outside the scope of this work.

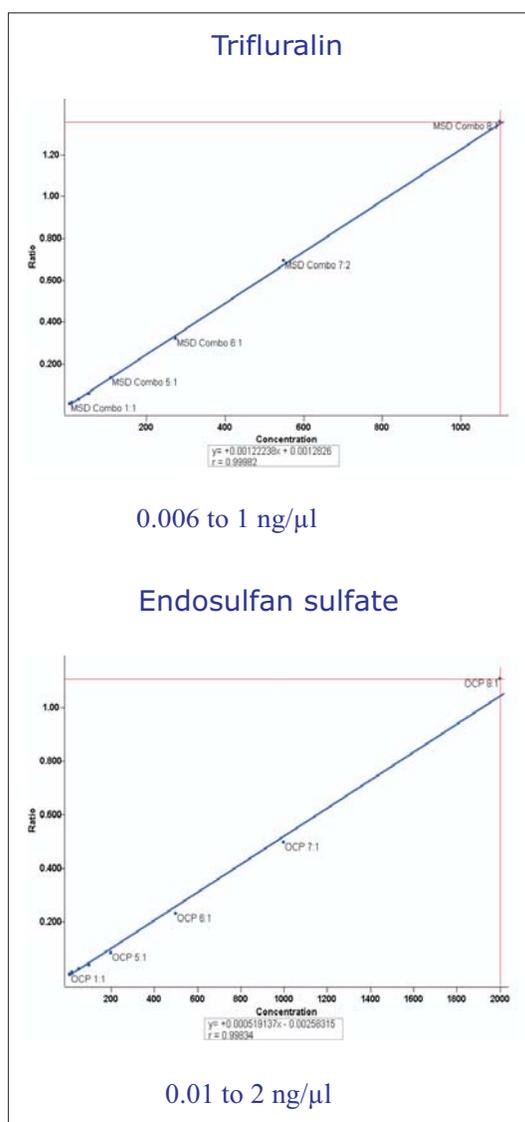


Figure 10. TOFMS calibration curves for Trifluralin and Endosulfan sulfate.

### Results for Pesticide Spikes

The surrogate recoveries for all matrices were around 90-100%, indicating efficient handling of the samples. Tables 1-5 summarize the quantitative pesticide results from the extract spikes. It is important to note again that pesticide spiking occurred after an extract was prepared. The goal of the work was to test the TOFMS proficiency to locate and quantify pesticides, not the efficiency of the sample preparation method.

#### Table Key

Det Freq = Detection Frequency (%) of pesticide in the listed commodity as noted in the Pesticide Data Program Annual Summary for 2000

EPA Tol = EPA Tolerance in ppm

Spike = Spike of pesticide in ppm

TOF = Measured by TOFMS (after spike) in ppm

Bkgd = Measured by TOFMS (before spike) in ppm

In summary, the spike and TOFMS measured values are very close for almost all of the pesticides. Several pesticides including Captan, Iprodione, Methomyl, and Oxamyl were quantified on the basis of their thermal degradation products. The carbamates Methomyl and Oxamyl do not usually survive as parent compounds in GC analysis. Captan and Iprodione, which were measured as their parent compounds in previous work where the GC run was only 13 minutes, likely degraded due to the slightly longer GC oven program (longer to facilitate elution of some of the pyrethroids and the Dimethomorphs).

**Table 1. Spike results for seedless red grapes.**

Pesticide	Det Freq	EPA Tol	Spike	TOF	Bkgd
Captan	28.1	50.0	0.40	0.66	ND
Iprodione	27.9	60.0	2.0	0.59	ND
Myclobutanil	20.5	1.0	0.065	0.066	ND
Omethoate	13.9	1	0.20	0.36	ND
Dimethoate	11.9	1	0.20	0.26	ND
Chlorpyrifos	9.0	0.5	0.020	0.022	ND
Methomyl	5.5	5	0.20	0.15	ND
Tebuconazole	4.7	5.0	0.65	0.75	ND
Carbaryl	3.9	10	0.20	0.32	ND
Dicloran	2.7	10	0.20	0.21	ND
Phosmet	1.6	10	0.20	0.27	ND
Piperonyl butoxide	1.3	8.0	0.35	0.39	ND
Diazinon	1.2	0.75	0.020	0.020	ND
Diazinon o analog	0	NT	0.025	0.042	ND
Propargite	1.2	10	0.65	0.23	ND
Propargite	1.2	10	0.65	0.51	ND
Azinphos methyl	0.7	4.0	0.20	0.34	0.09
Fenhexamid	NL	NL	0.20	0.30	ND

ND = not detected.

NT = no tolerance level set for the pesticide/commodity pair.

NL = not listed in PDP Annual Summary, but known to be used on the commodity.

**Table 2. Spike results for navel oranges.**

Pesticide	Det Freq	EPA Tol	Spike	TOF	Bkgd
Imazail	64.4	10.0	1.0	1.3	0.16
Thiabendazole	29.7	10	1.0	1.6	0.18
o-Phenylphenol	4.4	10.0	0.35	0.35	ND
Carbaryl	2.0	10	0.20	0.36	ND
Methidathion	1.5	2.0	0.20	0.28	ND
Chlorpyrifos	1.1	1.0	0.20	0.18	0.01

ND = not detected.

**Table 3. Spike results for red leaf lettuce.**

Pesticide	Det Freq	EPA Tol	Spike	TOF	Bkgd
Acephate	15.1	10	2.0	2.1	ND
cis-Permethrin	8.3	20.0	0.08	0.62	0.48
trans-Permethrin	8.3	20.0	0.08	0.49	0.36
Dimethoate	7.7	2	0.20	0.22	ND
Endosulfan sulfate	7.0	2.0	0.20	0.19	ND
Methamidophos	5.3	1.0	0.20	0.13	ND
Methomyl	2.8	5	0.20	0.24	ND
lambda-Cyhalothrin	2.5	2.0	0.50	0.68	ND
Diazinon	2.4	0.7	0.020	0.020	ND
Diazinon o analog	0	NT	0.025	0.035	ND
DCPA	2.2	2	0.20	0.23	0.01
Endosulfan I	2.2	2.0	0.20	0.19	ND
Endosulfan II	2.2	2.0	0.20	0.14	ND
Omethoate	1.9	2	0.20	0.12	ND
DDE	1.2	0.5	0.020	0.029	0.01

ND = not detected.

NT = no tolerance level set for the pesticide/commodity pair.

**Table 4. Spike results for bunch carrots.**

Pesticide	Det Freq	EPA Tol	Spike	TOF	Bkgd
Trifluralin	56.5	1.0	0.055	0.091	0.036
DDE	22.3	3	0.20	0.18	ND
Linuron	10.9	1	0.20	0.37	ND
DDT	6.9	3	0.20	0.18	ND
Diazinon	5.9	0.75	0.020	0.018	ND
Diazinon o analog	0	NT	0.025	0.037	ND
Metalaxyl	4.3	0.5	0.035	0.035	ND
Parathion	3.3	NT	0.20	0.22	ND
Phosmet	3.3	NT	0.17	0.32	ND
Dieldrin	2.7	0.1	0.020	0.024	ND
gamma-Chlordane	2.3	0.1	0.020	0.023	ND
alpha-Chlordane	2.3	0.1	0.020	0.023	ND
Carbaryl	2.2	10	0.20	0.30	ND
Parathion methyl	1.1	1	0.20	0.25	ND

ND = not detected.

NT = no tolerance level set for the pesticide/commodity pair.

**Table 5. Spike results for cucumber.**

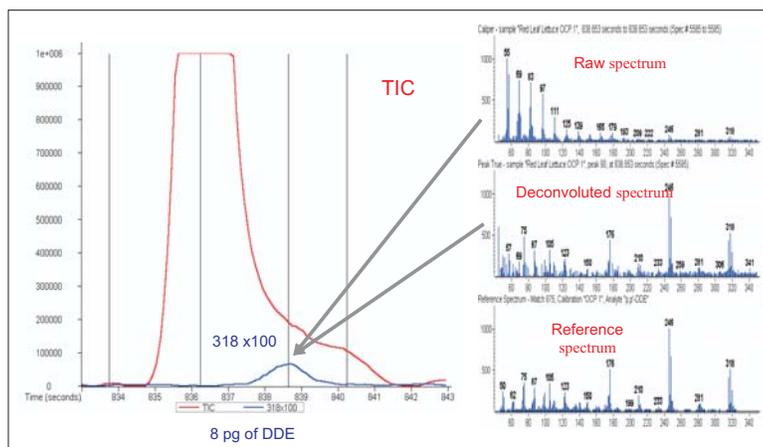
Pesticide	Det Freq	EPA Tol	Spike	TOF	Bkgd
Endosulfan sulfate	55.4	2.0	0.20	0.30	0.09
Endosulfan I	43.7	2.0	0.20	0.29	0.09
Endosulfan II	43.7	2.0	0.20	0.33	0.11
Dieldrin	16.3	0.1	0.020	0.026	ND
Metalaxyl	12.8	1.0	0.35	0.46	0.05
Methamidophos	11.5	1.0	0.20	0.21	ND
Chlorothalonil	9.4	5	0.25	0.53	ND
Oxamyl	3.1	2.0	0.20	0.12	ND

ND = not detected.

Note: 2 ppm of pesticide in commodity translates to 2 ng/ $\mu$ l of pesticide in extract based on the chosen sample preparation method. Therefore, 0.2 ppm = 200 pg/ $\mu$ l, and 0.02 ppm = 20 pg/ $\mu$ l.

#### Peak Find and Spectral Deconvolution in Matrix

A good example of locating a small peak in the presence of a big peak is given by the coelution of DDE and a large interfering compound in the lettuce extract (Figure 11). Almost certainly, a thousand-fold difference exists in the concentrations of these compounds, with DDE ultimately measured at only 8 pg/ $\mu$ l in the extract. The coeluted spectrum, taken at the DDE retention time, almost solely represents the interfering component; you can barely see the presence of 246 and 318 m/z ions, ions characteristic of DDE. Fortunately, the ChromaTOF software not only locates the peak, but also produces a deconvoluted mass spectrum that is fully library searchable and gives an excellent match of 875 versus a reference spectrum for DDE.



**Figure 11. Automated Peak Find and spectral deconvolution for DDE in lettuce. Note that the 318 ion for DDE is magnified by 100 so that it would appear above the baseline. The raw mass spectrum taken at the retention time for DDE signifies the huge interfering compound, but the deconvoluted spectrum matches well with a DDE reference spectrum.**

#### 4. Conclusions

Using a fast sample preparation method and fast GC-TOFMS, it is possible to quickly screen for hundreds of pesticides in fruit and vegetable extracts to determine whether EPA tolerances have been violated or even approached within an order-of-magnitude. Full mass spectra, even at low pg levels, provide excellent confirmation for a pesticide in a commodity extract. Peak find and deconvolution enable location and identification of pesticides, even those not targeted for Quantification, in fruits and vegetables.

#### 5. References

- United States Department of Agriculture, Pesticide Data Program Annual Summary Calendar Year 2000  
 S.J. Lehotay, M. Anastassiades, D. Stajnbaher, and F.J. Schenck, Quick, Easy Cheap, Effective, Rugged, and Safe (QuEChERS) Approach for the Determination of Pesticide Residues, 2002 Florida Pesticide Residue Workshop. <http://www.doacs.state.fl.us/~fs-prw/2002presentations.htm>  
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