# A Blind Study of Pesticide Residues in Spiked and Unspiked Fruit Extracts Using Deconvolution Reporting Software

Application

**Food**


## Author

Christopher P. Sandy
Agilent Technologies, Inc.
Block A, CSC Eskdale Road
Winnersh Triangle Estate
Wokingham, Berk RG41 5DZ
United Kingdom



## Abstract

**The Agilent Technologies mass selective detector (MSD) coupled with deconvolution reporting software (DRS) provides additional powerful data processing capabilities to the MSD ChemStation software. Reviewing full scan gas chromatography/mass spectrometry data for the confirmation of pesticide residues can be a labor-intensive and time-consuming process requiring great skill and concentration by an experienced analyst. The DRS is able to process a complex food extract total ion chromatogram in about 1 minute, whereas an experienced analyst may take more than 30 minutes to achieve the same quality result. Extensive data shown in this report supports the high confidence level that an analyst can have in results rapidly produced by the DRS.**


## Introduction

Typical mass spectral pesticide residue analysis requires finding target ions and meeting qualifier ion ratios. It is sometimes very difficult to confirm target compounds from high matrix background because the matrix affects the ion ratios of the target compounds or complicates the spectrum with additional ions. To be certain of the results, background subtraction and manual integration are often practiced. It is, therefore, a time-consuming process to confirm target compounds in a dirty matrix. It can take an experienced analyst 15 to 30 minutes to review/confirm one data file.

Two powerful gas chromatography/mass spectrometry (GC/MS) techniques - Retention Time Locking (RTL) and deconvolution were combined to create a quantitation and screening tool that can identify 567 pesticides and endocrine disrupters from a single run in 1–2 minutes. The Agilent Technologies GC/MSD-DRS provides the additional functionality to the MSD ChemStation.

## Experimental

### DRS Overview

A detailed overview of the DRS is given in an application note 5989-1157EN [1], available for download at www.agilent.com/chem. The operating principles of the DRS appear in Figure 1.

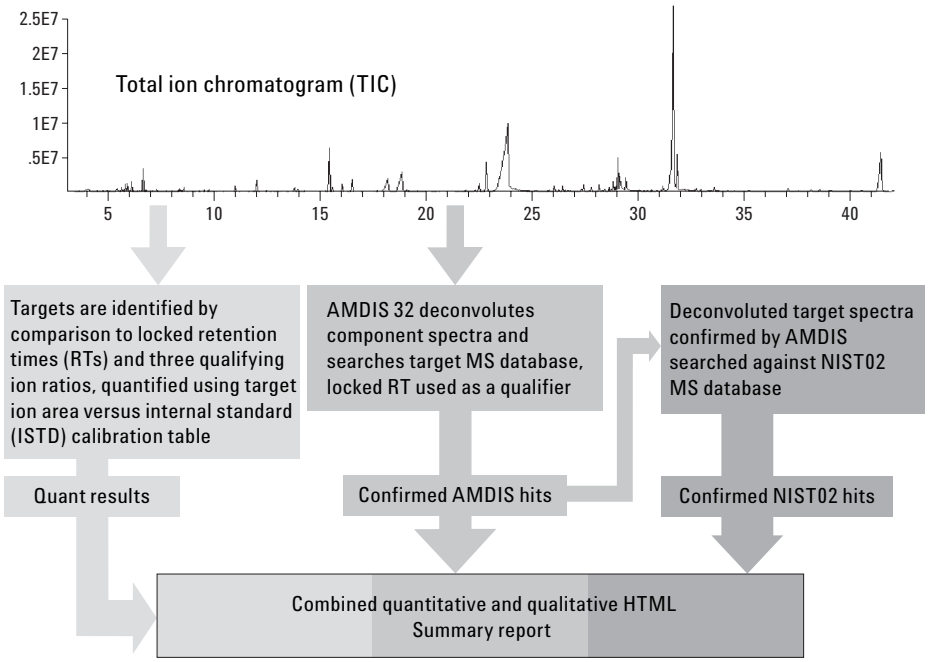


**Figure 1. Schematic diagram summarizing the GC/MS DRS.**

The quantitation capabilities of the MSD ChemStation are combined with the deconvolution power of the industry standard AMDIS program from NIST. AMDIS is able to separate spectra of interest from dirty matrix spectra present in samples analyzed for pesticides. A third level of confidence is obtained by sending the deconvoluted spectra for library searches of the NIST02 145,000 compound library. A comprehensive report is produced in about 1 minute.

## Samples

Six samples of fruit extracts, supplied in 90/10 iso-octane/toluene solvent were received for analysis by GC/MS. The samples were prepared by an accredited food pesticide laboratory based in Scandinavia. Three of the samples were spiked with a number of pesticides at varying concentration levels. Although the range of concentrations of the pesticides in each sample was given, **neither the actual number of pesticides** spiked into each control sample **nor the identities were** supplied. Details of the samples appear in Table 1. The other three samples were 'real', unspiked extracts.

**Table 1. Sample Details for Blind Study**

| Sample number | Matrix extracted | Number of pesticides | Concn range (mg/Kg) | Comments |
|---|---|---|---|---|
| 1 | Orange | 20–40 | 0.02–0.20 | Control sample - spiked |
| 2 | Lettuce | 20–40 | 0.02–0.20 | Control sample - spiked |
| 3 | Apple | 20–40 | 0.01–0.20 | Control sample - spiked |
| 4 | Grapes | 2–4 | 0.1–1.0 | Real sample |
| 5 | Orange | 2–4 | 0.2–5.0 | Real sample |
| 6 | Apple | 2–4 | 0.05–2.0 | Real sample |

## Instrumentation

The samples were analyzed by full-scan GC/MS using the analytical conditions given in Table 2. Data processing and reporting were performed using the default settings provided with the DRS.

**Table 2. RTL GC/MS Analysis Conditions for Fruit Extract Samples**

| **Gas chromatograph** | **Agilent 6890N** |
|---|---|
| Column | 30 m x 0.25 mm id x 0.25 µm HP-5MS (p/n 19091S-433) |
| Carrier gas | Helium |
| Flow rate | 1.9 mL/min at 70 °C |
| Head pressure | 18 psig, constant pressure mode<br>Method RTLocked to methyl chlorpyrifos at 16.593 min |
| Injector type | PTV, septumless head |
| Injector temperature (°C), hold time (min), and ramp rate (°C/min) | 90 °C (0.3 min) - 1720 °C/min - 250 °C |
| Vent time | 0.2 min |
| Vent flow | 30 mL/min |
| Vent pressure | 0 psig |
| Purge flow | 60 mL/min |
| Purge time | 1.0 min |
| Syringe volume | 50 µL |
| Injection volume | 15 µL |
| Liner | Empty multibaffle |
| Oven program: temperature (°C), hold time (min), and ramp rate (°C/min) | 70(2)-25-150(0)-3-200(0)-8-280(10) |
| **MSD** | **Agilent 5973 inert** |
| MS interface | 280 °C |
| MS source | 230 °C |
| MS quad | 150 °C |
| Detection mode | EI, Scan 40–550 amu |
| EM voltage | ATUNE value |

## Results

The results for the three spiked extracts appear in Table 3 - note that the details of which pesticides were added to the spiked samples were not supplied until after the results were shown to the customer. Those pesticides confirmed by the DRS, are shown lightly shaded. The analytes, shown darkly shaded, are not present in the Agilent RTL Pesticides database. Analyte entries left unshaded were not confirmed.

**Table 3. MSD-DRS Results for Three Spiked Fruit Extract Samples**

| | Sample 1: Control-orange, spiked | | Sample 2: Control-lettuce, spiked | | Sample 3: Control- apple, spiked | |
|---|---|---|---|---|---|---|
| | Pesticide | Added mg/kg | Pesticide | Added mg/kg | Pesticide | Added mg/kg |
| 1 | Methamidofos* | 0.10 | Diphenylamine | 0.10 | Mevinphos | 0.05 |
| 2 | Dichlorvos* | 0.10 | HCB | 0.02 | Trichlorfon | 0.05 |
| 3 | Acephate* | 0.10 | Lindane (HCH-gamma) | 0.04 | Heptenophos | 0.02 |
| 4 | Omethoate | 0.10 | Diazinon | 0.04 | Tecnazene | 0.01 |
| 5 | Propachlor | 0.20 | Chlortalonil | 0.04 | HCH alpha | 0.01 |
| 6 | Chlorprofam | 0.10 | Vinclozolin | 0.04 | HCH beta | 0.02 |
| 7 | Monocrotophos | 0.10 | Carbaryl | 0.20 | Dichloran | 0.05 |
| 8 | Dimethoate | 0.04 | Metalaxyl | 0.10 | Pyrimethanil | 0.02 |
| 9 | Quintozene | 0.02 | Pirimiphos-methyl | 0.10 | Etrimphos | 0.02 |
| 10 | Parathion-methyl | 0.10 | Malathion | 0.10 | Ethiofencarb | 0.10 |
| 11 | Dichlofluanid | 0.10 | Chlorpyrifos | 0.10 | Metribuzin | 0.05 |
| 12 | Fenpropimorph | 0.10 | Cyprodinil | 0.04 | Toclophos methyl | 0.01 |
| 13 | Triadimefon | 0.04 | Penconazole | 0.04 | Linuron | 0.05 |
| 14 | Thiabendazole | 0.10 | Captan | 0.10 | Aldrin | 0.02 |
| 15 | Tolylfluanid | 0.04 | Folpet** | 0.10 | Diethofencarb | 0.02 |
| 16 | Mecarbam | 0.10 | Procymidone | 0.04 | Trichloronate | 0.02 |
| 17 | Methidation | 0.10 | Endosulfan-a | 0.04 | Triadimenol | 0.05 |
| 18 | Vamidothion | 0.10 | pp-DDE | 0.04 | Disulfoton sulfoxide | 0.20 |
| 19 | Imazalil | 0.10 | Bupirimate | 0.04 | Disulfoton sulfone | 0.02 |
| 20 | Myclobutanil | 0.10 | Endosulfan-b | 0.04 | Fluazinam | 0.05 |
| 21 | Kresoxim methyl | 0.10 | Aclonifen | 0.04 | Chlorbenzilate | 0.05 |
| 22 | Tebuconazole | 0.10 | Ethion | 0.04 | Oxadixyl | 0.05 |
| 23 | Phosmet | 0.10 | Triazophos | 0.04 | Benalaxyl | 0.05 |
| 24 | Fenpropathrin | 0.04 | Endosulfan-sulfate | 0.04 | Dicofol | 0.05 |
| 25 | Tetradifon | 0.04 | Iprodione | 0.04 | Fenazaquin | 0.02 |
| 26 | Azinphos-methyl | 0.10 | Bromopropylate | 0.10 | Pyrazophos | 0.05 |
| 27 | Fenarimol | 0.10 | Methoxychlor | 0.10 | Acrinathrin | 0.02 |
| 28 | Azinpfos-ethyl | 0.10 | Phosalone | 0.10 | Bitertanol | 0.05 |
| 29 | Prochloraz | 0.10 | Lambda-Cyhalothrin | 0.04 | Cyfluthrin beta | 0.05 |
| 30 | Flucythrinate | 0.10 | Permethrin | 0.10 | Alpha cypermethrin | 0.05 |
| 31 | Esfenvalerate | 0.04 | Cypermethrin | 0.10 | | |
| 32 | Azoxystrobin | 0.04 | Fenvalerate | 0.04 | | |
| 33 | | | Deltamethrin | 0.10 | | |

* See Discussion item 1.

** See Discussion item 2.

The results for the three 'real' extracts appear in Table 4. Those pesticides confirmed by the DRS are shown lightly shaded. The darkly-shaded analytes are not present in the Agilent RTL Pesticides database. Analyte entries left unshaded were not confirmed. Analytes with an associated concentration were confirmed as present by the customer using NPD/ECD. Lightly-shaded analytes without a concentration label were detected and confirmed by the DRS, but not by the customer.

**Table 4. MSD-DRS Results for Three 'Real' Fruit Extract Samples**

| |
|---|
| **Sample 4: Grapes** |
| 0.68 mg/Kg Captan |
| 0.21 mg/Kg Cyprodinil |
| 0.27 mg/Kg Fludioxinil |
| Diphenylamine |
| **Sample 5: Orange** |
| 2.5 mg/Kg Imazalil |
| 0.25 mg/Kg Medidathion |
| 3.0 mg/Kg Thiabendazole |
| **Sample 6: Apple** |
| 0.86 mg/Kg Diphenylamine |
| 0.05 mg/Kg Chlorpyrifos |
| 0.79 mg/Kg Thiabendazole |
| Dimethoate |
| Ethoxyquin |
| Methyl parathion |
| Endosulfan sulfate |
| Propargite |

## Discussion

1. Control - Orange spiked extract

This control sample was spiked with 32 pesticides at levels ranging between 0.02 and 0.10 mg/kg. Twenty-six pesticides were detected and confirmed by the DRS software, two were not reported since they are not present in the Agilent RTL Pesticide database and four were not detected. The spiking was done to the raw matrix, not to a matrix extract. For the polar pesticides (methamidofos and acephate), the recovery was in the 20%–30% range as confirmed by NPD/ECD. Therefore, that explains why these pesticides were not detected by DRS.

2. Control - Lettuce spiked extract

This control sample was spiked with 33 pesticides at levels ranging between 0.02 and 0.20 mg/kg. Twenty-nine pesticides were detected and confirmed by the DRS software, three were not reported since they are not present in the Agilent RTL Pesticide database and one was not detected. The one undetected analyte, (Folpet, marked with two asterisks in Table 3), was detected and confirmed if a higher sensitivity setting was used in the AMDIS deconvolution program.

3. Control - Apple spiked extract

This control sample was spiked with 30 pesticides at levels ranging between 0.01 and 0.20 mg/kg. Twenty-two pesticides were detected and confirmed by the DRS software, six were not reported since they are not present in the Agilent RTL Pesticide database and two were not detected.

Overall, of the 95 spiked analytes in the three control samples, 93% of the pesticides present in the Agilent RTL Pesticide database were detected and confirmed by full-scan library searching of the deconvoluted mass spectra.

4. 'Real' Grape extract

The customer had detected and confirmed three pesticide residues in the Grape extract sample - Captan, Cyprodinil, and Fludioxinil. Of these three analytes, Captan was confirmed by the DRS and Cyprodinil and Fludioxinil are not entries in the Agilent RTL Pesticide database. However, DRS also confirmed an additional pesticide residue - Diphenylamine, which was not reported by the customer.

5. 'Real' Orange extract

The customer had detected and confirmed three pesticide residues in the Orange extract sample - Imazilil, Methidathion, and Thiabendazole. All three of these pesticides were confirmed by the DRS software and no other analytes were confirmed.

6. 'Real' Apple extract

The customer had detected and confirmed three pesticide residues in the Apple extract sample - Diphenylamine, Chlorpyriphos, and Thiabendazole. All three of these pesticides were confirmed by the DRS. In addition, the DRS also confirmed the presence of five additional pesticide residues - Dimethoate, Ethoxyquin, Methyl Parathion, Endosulfan Sulfate, and Progargite. These five pesticides had not been reported by the customer.

## Conclusions

The Agilent Technologies MSD-DRS provides additional powerful data processing capabilities to the MSD ChemStation software. Reviewing full scan GC/MS data for the confirmation of pesticide residues can be a labor-intensive and time consuming process requiring great skill and concentration by an experienced analyst.

The DRS is able to process a complex food extract TIC in the order of 1 minute, whereas an experienced analyst may take more than 30 minutes to achieve the same quality result. The DRS software was proven to report the lowest number of false positives and false negatives in the shortest time period.

In scan mode, the detection limit is not as low as in selected ion monitoring (SIM) mode; however, any prior knowledge of the target analytes (retention times or characteristic ions) is not required for the DRS.

The extensive data shown in this report, run under totally blind conditions, shows the high degree of confidence that an analyst can have in the results produced by the DRS in minutes.

## Reference

1. Philip L. Wylie, Michael J. Szelewski, Chin-Kai Meng, and Christopher P. Sandy, "Comprehensive Pesticide Screening by GC/MSD Using Deconvolution Reporting Software", Agilent Technologies, publication 5989-1157EN, www.agilent.com/chem

## For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.



Printed in the USA
October 5, 2004
5989-1654EN