# Reducing Analysis Time Using GC/MSD and Deconvolution Reporting Software

Application

**Food and Flavors**


## Authors

Mike Grady, Steve Morrison, and Bob Deets
Campbell Soup Company
Campbell Place
Camden, NJ 08103
USA

Mike Szelewski
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA



## Abstract

**Analyzing a complex matrix can be accomplished using multiple specific detectors, but a significant time savings is realized using GC/MSD. Using an available Retention Time Locked database with Deconvolution Reporting Software (DRS) adds a second "expert" opinion. GC/MSD with DRS provides the fastest methodology to the fewest number of false positives/negatives with the greatest confidence in the results.**


## Introduction

Analyzing a complex matrix can be accomplished using multiple specific detectors such as electron capture (ECD), nitrogen-phosphorus (NPD), and dual flame photometric for phosphorus and sulfur (DFPD). Most of the analytes, depending on their molecular formula, could be run on two of these specific detectors for confirmation. However, that would take twice the time. A second choice is to run each sample on unlike columns of differing polarities to the same type detector. Both of these approaches, using ECD, NPD, and/or DFPD, involve more sample handling/tracking, usually twice the number of runs and subsequent data interpretation, and still only have retention time as an identifier. Some methodologies require final confirmation by GC/MSD. Also, it is very difficult to analyze for hundreds of compounds using a non-MS method due to peak overlaps.

Campbell Soup Company has been moving from GC-specific detector analyses to GC/MSD analyses for the above-mentioned reasons. A significant time savings can be realized using GC/MSD. It is imperative, however, that data quality be maintained while increasing productivity.

Agilent Technologies recently introduced Deconvolution Reporting Software (DRS) for use with a GC/MSD system [1]. DRS automatically combines the results from the MSD ChemStation Quantitation with AMDIS Deconvolution and NIST Search into an easy-to-read report. Using an available Retention Time Locked database reduces methods development for DRS and speeds data comparison among labs. Positives found by normal quantitation are confirmed, and the analyst is directed to further target compound hits to verify. The analyst

need not spend the time to review hundreds of possible compounds, which could take hours per sample. GC/MSD with DRS provides the fastest methodology to the fewest number of false positives/negatives with the greatest confidence in the results. It typically takes less than 2 minutes to process a DRS Report.

The purpose of this application note is to show the use of GC/MSD with DRS on complex samples in Campbell's Research Lab. It is also intended to show a time savings while maintaining data quality.

## Experimental

### Instrument Operating Parameters

The instrument operating parameters are listed in Table 1. These conditions may have to be optimized for use in another laboratory.

A Programmable Temperature Vaporizing inlet (PTV) is used in the solvent vent mode (SV). The sample is injected at or below the boiling point of the solvent, in this case 50 °C. Solvent is evaporated through the split vent line with helium at 200 mL/min for 0.3 min. At 0.5 min, the PTV is rapidly heated to 320 °C, transferring the analytes, with minimum solvent, onto the column. The column is held at the initial temperature of 70 °C during this process. The PTV-SV allows larger volumes of sample to be injected, 10 µL for this study, versus the typical 1 µL for this column.

The PTV inlet liner, 5183-2037, is multi-baffled and deactivated. It does not contain glass wool, which could contribute to active compound degradation. This liner has sufficient capacity to accommodate the 10 µL injection volume

The HP-5MS column was used by Agilent to develop the original method and is run in constant pressure mode. Constant pressure methods can be precisely scaled, or sped up, for faster analyses. The retention times of 927 analytes have been recorded on this column.

The system is Retention Time Locked to methyl chlorpyrifos at 16.596 min. The primary benefit of RTL for a food laboratory is the ability to maintain retention times after clipping or changing the column. Other benefits include: 1) constant quantitation database and integration events times; 2) switching group times remain constant for laboratories performing SIM analyses; 3) multi-site laboratories can easily compare data; 4) commercially available RTL databases can be used. Additional information is available at www.agilent.com/chem, with application notes detailing the numerous benefits of RTL.

The injector parameters are modified from the default "Fast Injection" to "Variable". This allows matching the injector parameters to the PTV-SV requirements. Most importantly, the injection speed is slowed to accommodate the evaporation of the solvent during injection.

The 5973N MSD had been upgraded to an inert source and Performance Electronics. The inert source has shown improved response and less degradation for active compounds. Performance electronics minimize noise at faster Scan sampling rates and allow shorter dwell times and more ions/group for SIM acquisitions.

A sampling rate of 2 and a scan range of 35-500 yields 3.12 scans/sec. This results in at least 10 data points across the earliest narrow peaks that are 0.055 min wide. This is a good number of points for both quantitation and deconvolution while minimizing noise.

**Table1. Gas Chromatograph and Mass Spectrometer Conditions**

| | | | |
|---|---|---|---|
| **GC** | **Agilent Technologies 6890N** | | |
| **Inlet** | EPC PTV | | |
| Mode | Solvent vent | | |
| Temperature ramp | °C/min | Next °C | Hold min |
| Initial | | 50 | 0.50 |
| Ramp 1 | 600 | 320 | 3.00 |
| Ramp 2 | 50 | 200 | 0.00 |
| Cryo | On | | |
| Cryo use temperature | 50 °C | | |
| Cryo timeout | 10.00 min (On) | | |
| Cryo fault | On | | |
| Pressure | 17.98 psi (On) | | |
| Vent time | 0.30 min | | |
| Vent flow | 200.0 mL/min | | |
| Vent pressure | 0.0 psi | | |
| Purge flow | 400.0 mL/min | | |
| Purge time | 1.00 min | | |
| Total flow | 403.9 mL/min | | |
| Gas saver | On | | |
| Saver flow | 20.0 mL/min | | |
| Saver time | 2.00 min | | |
| Gas type | Helium | | |
| **PTV liner** | Agilent PTV Liner part# 5183-2037 | | |
| **Oven** | **120 V** | | |
| Oven ramp | °C/min | Next °C | Hold min |
| Initial | | 70 | 2.00 |
| Ramp 1 | 25 | 150 | 0.00 |
| Ramp 2 | 3 | 200 | 0.00 |
| Ramp 3 | 8 | 280 | 10.00 |
| Total run time | 41.87 min | | |
| Equilibration time | 0.5 min | | |
| Oven max temperature | 325 °C | | |
| **Column** | Agilent Technologies HP 5 MS, part# 19091S-433 | | |
| Length | 30.0 m | | |
| Diameter | 0.25 mm | | |
| Film thickness | 0.25 µm | | |
| Mode | Constant Pressure | | |
| Pressure | 17.98 psi | | |
| Nominal initial flow | 1.9 mL/min | | |
| Inlet | Front | | |
| Outlet | MSD | | |
| Outlet pressure | Vacuum | | |
| **RTL** | System Retention Time Locked to methyl chlorpyrifos at 16.596 min | | |
| **Front injector** | | | |
| Sample washes | 0 | | |
| Sample pumps | 3 | | |
| Injection volume | 10.00 µL | | |
| Syringe size | 25.0 µL | | |
| PreInj Solv A washes | 0 | | |
| PreInj Solv B washes | 1 | | |
| PostInj Solv A washes | 2 | | |
| PostInj Solv B washes | 2 | | |
| Viscosity delay | 1 s | | |
| Plunger speed | Variable | | |
| Injection speed | 50.00 µL/min | | |
| Draw speed | 600.00 µL/min | | |
| Dispense speed | 1000.00 µL/min | | |
| PreInjection dwell | 0.00 min | | |
| PostInjection dwell | 0.00 min | | |
| **MSD** | Agilent Technologies 5973N | | |
| Upgrades | Inert source and Performance Electronics | | |
| Solvent delay | 4.00 min | | |
| Low mass | 35 amu | | |
| High mass | 500 amu | | |
| Threshold | 50 | | |
| Sampling | 2 | | |
| Scans/sec | 3.12 | | |
| Quad temperature | 150 °C | | |
| Source temperature | 230 °C | | |
| Transfer line temp | 280 °C | | |
| Tune Type | Autotune | | |

**Calibration Standards**

Prepared from certified reference standards available from Chem-Serve and Crescent Chemical Company. All standards were corrected for purity.

### Extraction Procedure

An appropriate amount of commodity is weighed, typically 10-15 grams. Surrogates and, if necessary, fortification standards (spike) are added. The commodity is extracted with 1% acetic acid in acetonitrile, centrifuged [2], and passed through an SPE cartridge [3]. Analytes are eluted from the cartridge using acetonitrile/toluene. A 1-gram volume equivalent is taken from the eluant and internal standard(s) is added. The extract is brought to near dryness and solvent exchanged into ethyl acetate for GC/MSD analysis.

## Results

Six commodities - apples, lettuce, carrots, celery, green peppers, strawberries, and tomatoes - were purchased at local supermarkets. They were extracted and analyzed using the GC/MSD conditions described earlier. Aldrin was added to each during the extraction process and acts as both a surrogate standard (SS) and an internal standard (IS).

The datafiles were processed using Agilent's Deconvolution Reporting Software. DRS automatically combines the results from the MSD ChemStation Quantitation with AMDIS Deconvolution and NIST Search into an easy-to-read report.

The results are shown in Table 2. Each of the samples showed at least one residue, ranging from trace quantities (< 0.02 ppm) to 0.48 ppm. In most cases, the trace quantities were not found by the GC/MSD standard quantitation using 3 qualifier ion identification.

As a verification of the methodology, a second sample of strawberries and tomatoes were each fortified with six analytes at the 0.1 ppm level, together with the Aldrin SS/IS. The analyses' results for these spiked samples are shown in Table 3. There are excellent recoveries for most analytes. Responses for two analytes in tomato, atrazine, and permethrin were higher than expected and could be due to matrix enhancement during injection. Time constraints did not allow for further investigation.

Duplicate results are also shown in Table 3. The chlorothalonil in tomato at 0.08 ppm compares favorably with the 0.09 ppm found in the first sample. The same is true for captan at 0.47 ppm in the duplicate versus 0.48 ppm in the original sample.

The GC/MSD system was calibrated for more than 50 compounds. Using DRS, the analyst can get a verification of the presence of those 50 compounds together with an automated "expert second opinion" of other compounds that may be present. This second opinion is in two distinct parts. First, the deconvolution of the complex TIC with subsequent matching of clean spectra to a database is provided. Second, the matching of these clean spectra to an independent database, in this case the NIST05a library of > 163,000 compounds.

**Table 2. Market Basket Commodity Results**

| Commodity Compound | Table R.T. | Apple | Lettuce | Carrot | Celery | Gr pepper | Strawberry | Tomato |
|---|---|---|---|---|---|---|---|---|
| Methamidopho | 5.655 | | | | | 0.04 | | |
| Acephate | 7.690 | | t | | | 0.23 | | |
| Diphenylamine | 10.516 | t | | | | | | |
| Chlorothalonil | 14.784 | | | | 0.02 | t | | 0.09 |
| Carbaryl | 16.806 | 0.02 | | | | | | |
| Metalaxyl | 17.337 | | | | | | t | |
| Malathion | 18.800 | | | | t | | | |
| Isodrin | 20.031 | | | t | | | | |
| Thiabendazole | 20.939 | 0.02 | | | | | | |
| Captan | 21.227 | | | | | | 0.48 | |
| Phosmet | 28.504 | 0.03 | | | | | | |
| Permethrin | 31.369* | t | | | 0.03 | | | t |
| Cyfluthrin | 32.218* | t | | | | | | |
| Cypermethrin | 32.690* | t | | | | | | |
| Fenvalerate | 34.271* | t | | | | | | |
| Addional DRS-only compounds | | 8 | 0 | 1 | 3 | 13 | 10 | 1 |

* First R.T. of multiple isomers. t = Trace quantity

**Table 3. Spiked and Duplicate Commodity Results, ppm**

| | | Spikes | |
|---|---|---|---|
| **Compound** | **Table R.T.** | **Strawberry** | **Tomato** |
| Atrazine | 13.159 | 0.15 | 0.25 |
| Lindane | 13.461 | 0.11 | 0.11 |
| Carbaryl | 16.806 | 0.11 | 0.15 |
| Linuron | 18.187 | 0.13 | 0.19 |
| Parathion | 19.275 | 0.09 | 0.13 |
| Permethrin I | 31.369 | 0.14 | 0.26 |
| Permethrin II | 31.550 | 0.15 | 0.25 |
| | | **Duplicates** | |
| Chlorthalonil | 14.784 | | 0.08 |
| Captan | 21.227 | 0.47 | |

A portion of the DRS Report for celery is shown in Figure 1. Chlorothalonil was found at 14.823 minutes at 0.02 ppm. AMDIS verified chlorothalonil with a 97 match eluting only 2.7 seconds from its expected RTL time. NIST search further verified chlorothalonil with a 93 match, as the third hit out of the top 100 hits. Aldrin and permethrin are similarly verified with permethrin below the normal reporting limit. Malathion was found by AMDIS and verified by NIST. It was not found by ChemStation because one of three qualifier ions was out of range. AMDIS mitigates this problem because deconvolved spectra are cleaned of interferences and full spectrum matching is used. It would be nearly impossible to identify the analytes of interest in the presence of > 650 individual components in the celery without deconvolution. The TIC for celery is shown in Figure 2.

For all of the commodities tested, DRS verified the presence of all the calibrated peaks found by the ChemStation. Most laboratories only calibrate a fixed number of compounds, say 50-100, as it is not practical to calibrate for all 927. At the same time, these laboratories are interested in identifying other compounds that may be present in samples. Numerous uncalibrated compounds were identified using the 927 compound DRS database. The number of these additional compounds is shown on the last line in Table 2, excluding phthalates, cresols, and sulfur. When these are important to the laboratory, the GC/MSD system can, of course, be calibrated using additional standards. If an estimate of the amount is needed, or if a standard is unavailable, an average response factor can be used. The DRS database provides an average response factor for all 927 compounds.

In contrast to the above methodology is the use of multiple element specific detectors such as ECD, NPD, and DFPD. Most of the analytes could be run on two of these specific detectors, but that would take twice the time. Also, it is very difficult to analyze for 927 compounds using a non-MS method due to peak overlaps. A second choice is to run each sample on unlike columns of differing polarities to the same type detector. Both of these approaches, using ECD, NPD, and/or DFPD, involve more sample handling/tracking, usually twice the number of runs, and still only have retention time as an identifier.

MSD Deconvolution Report
Sample Name: celery
Data File: C:\msdchem\1\Data\sjm03.D
Date/Time: 02:30 PM Friday, Jan 19 2007

The NIST library was searched for the components that were found in the AMDIS target library.

| | | | Agilent ChemStation | AMDIS | | NIST | |
|---|---|---|---|---|---|---|---|
| **R.T.** | **CAS #** | **Compound Name** | **Amount (ppm)** | **Match** | **R.T. Diff Sec** | **Reverse Match** | **Hit Num.** |
| 14.823 | 1897456 | Chlorothalonil | 0.02 | 97 | 2.7 | 91 | 3 |
| 18.5992 | 309002 | Aldrin | 1 | 99 | 4.3 | 93 | 1 |
| 18.7799 | 121755 | Malathion | | 59 | -1.2 | 47 | 1 |
| 31.3134 | 52645531 | Permethrin I | 0.03 | 71 | -3.3 | 53 | 5 |

**Figure 1. DRS report for celery.**

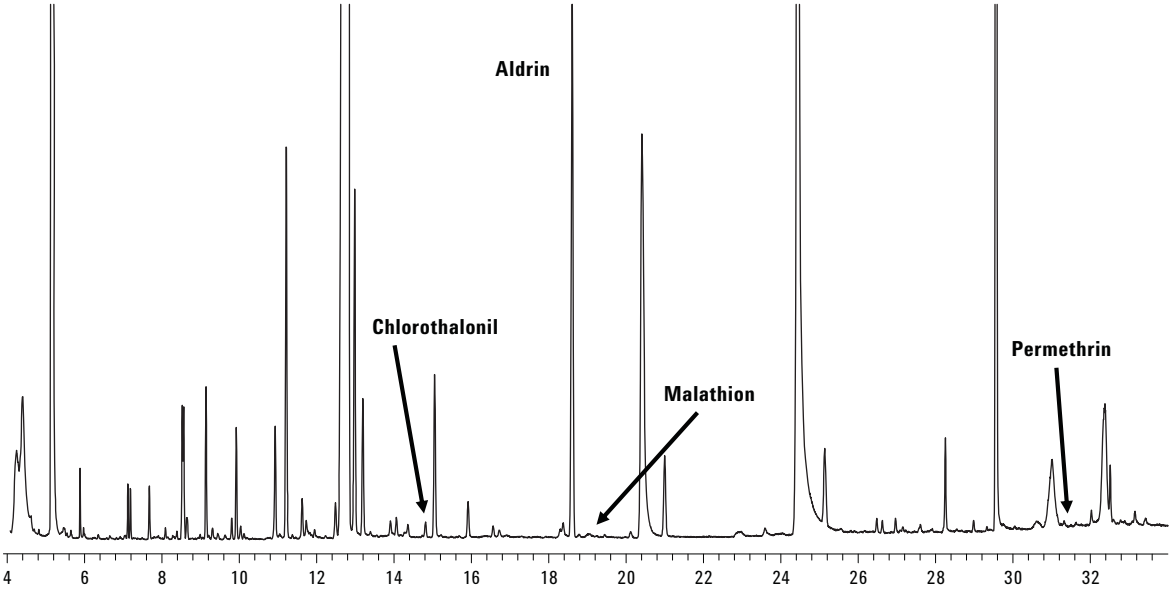


**Figure 2. Market basket celery total ion chromatogram.**

## Conclusions

Analyzing a complex matrix can be accomplished using multiple specific detectors, but a significant time savings is realized using GC/MSD. Many more analytes can be determined simultaneously using mass spectra for confirmation. Using an available Retention Time Locked database reduces methods development and speeds data comparison among labs. Deconvolution Reporting Software adds a second "expert" opinion. Positives found by normal quantitation are confirmed, and the analyst is directed to further target compound hits to verify. GC/MSD with DRS provides the fastest methodology to the fewest number of false positives/negatives with the greatest confidence in the results.

## References

1. "Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software," Philip L. Wylie, Michael J. Szelewski, and Chin-Kai Meng, Agilent Technologies Pub # 5989-1157EN.
2. "Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and 'Dispersive Solid-Phase Extraction' for the Determination of Pesticide Residues in Produce," M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck. (2003) J.AOAC Int. 86, 412-431.
3. "Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food," Japan Department of Food Safety, Ministry of Health, Labour and Welfare.

## For More Information

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



Printed in the USA
May 14, 2007
5989-6677EN