Application Note

Food Testing

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Pesticide Screening in Strawberries Using the Agilent 8860 GC with the Agilent 5977B GC/MSD and SureTarget Deconvolution

Authors

Anastasia A. Andrianova, Bruce D. Quimby, and Jessica L. Westland

Agilent Technologies, Inc.

Abstract

The Agilent 8860 GC with the Agilent 5977B GC/MSD system was used for the screening of pesticides in strawberries. This cost-effective system, combined with appropriate sample preparation, operating conditions, and software tools, provides a useful way to identify pesticides and other contaminants in complex matrices such as foods. The instrument configuration incorporated pulsed splitless injection, a stainless steel EI source, and retention time locking to a database of pesticides and environmental pollutants. Complete analysis was done in two steps. Samples were first screened using Agilent MassHunter Unknowns Analysis software, which provides automated deconvolution and library searching to identify any pesticides or other chemicals of concern. Based on the screening results, the sample was then analyzed to quantify any compounds of interest that were found. Samples of strawberries, purchased from local grocery stores, were used to demonstrate the capabilities of the method.

Introduction

Trace-level pesticide and environmental pollutants in the food supply remain a worldwide concern that is driving the demand for more rapid and reliable methods of analysis. The challenge is to find technologies that can search for hundreds of pesticides, polycyclic aromatic hydrocarbons (PAHs), and other targets in complex food matrices. Often, methods are aimed at a specific list of compounds that are commonly found in a food product. These methods can be effective, but may overlook residues that are not specifically targeted.

This approach is intended to find as many compounds of concern as possible using a multistep approach. The first step is to obtain mass spectral scan data on the samples with the GC/MSD system retention time locked (RTL) to a library of pesticides and environmental pollutants containing over 1,000 compounds. The scan data are then processed in Agilent MassHunter Quantitative 10 Unknowns Analysis software, which provides streamlined automated deconvolution and library searching. Previous approaches to processing scan data for library searching relied on comparing a baseline-subtracted apex spectrum of a peak to reference spectra. This approach can work well when there are no chromatographic interferences with the peak. Food samples, however, often contain significant levels of matrix compounds that can interfere with the process, making analyte identification challenging.

Spectral deconvolution is a long-used software approach to remove the ions of coeluting compounds from the spectrum of an analyte. In deconvolution, ion chromatograms are extracted at all masses in the scan range. Ions with chromatographic peaks having the same shape and retention time (RT) are grouped into components. The responses of ions present in multiple overlapping peaks are apportioned to each peak using a similar process to that in chromatographic integrators. Spectra are then constructed from the components. The deconvolution process greatly reduces or eliminates interfering ions in the analyte spectra.

MassHunter Quantitative 10 Unknowns Analysis software has a powerful set of tools to deconvolute the spectra in a scan file and search the components against libraries. Peaks with high library match scores are then inspected as possible hits. If the libraries contain RT or retention index (RI) information, these can be used to filter the search results and serve as further evidence of a compound's presence. Generally, the higher the library match score (LMS), and the closer the RT match, the more likely the compound is present. This screening is most effectively done with a spectral library containing RTs or RIs collected under RTL conditions and with scan data locked to the same time scale. With RTL, RTs usually match those of the library within 0.1 minutes or less. This Application Note assembled a spectral library of >1,000 compounds with RTs locked to the Agilent pesticides and environmental pollutants MRM database[1] and to the Agilent MassHunter pesticides Personal Compound Database and Library (PCDL) and workflow for GC/Q-TOF.[2] MassHunter Unknowns Analysis can automatically process a complete scan file in minutes, and produce a report of LMS and RT match data, which is then inspected to determine the compounds present.

Further screening can be done by searching the deconvoluted components against the NIST library. The NIST 17 library contains RIs experimentally determined on semistandard nonpolar columns of the type used here for many of the entries. An alkane RI calibration mix is run with the RTL pesticide method, and used to create an RI calibration file. MassHunter Unknowns Analysis then searches the deconvoluted spectra through NIST 17 and lists the LMS and RI values for hits as well as the NIST RI values, if available. This tool is very powerful, but because it searches all matrix components, it can lead to a very large list of hits to be inspected.

Once the list of compounds from screening the samples is determined, a separate method is created for quantifying those of interest as well as any others to be monitored.

To demonstrate the utility of this approach, 16 samples of strawberries were purchased from various grocery stores and farmer's markets around Cupertino, California, and subjected to analysis with the method. Strawberries often require the application of pesticides to successfully grow an acceptable product. The strawberry samples were extracted with a QuEChERS method resulting in extracts in acetonitrile as the solvent.

Given the active nature of many of the pesticides, the choice of inlet and injection technique should be optimized. In this case, pulsed hot splitless injection was found to provide good analytical results. Acetonitrile is not a solvent of choice for pulsed hot splitless injection into a GC with the columns used. There are often problems with poor peak shapes. This method addresses these problems using a low pressure drop (LPD) inlet liner and changing the initial oven temperature and hold time.

To prevent ghost peaks in subsequent runs from high-boiling matrix contaminants that elute after the analytes, an extended bake-out time was used. With continued use, the highest boiling contaminants can deposit in the head of the column, resulting in RT shifts, poor peak shape, and reduced response. This problem is addressed by trimming the head of the column and relocking the RTs with the RTL software tool.

Experimental

The system used in this work was configured to identify pesticides in strawberry extracts. The important techniques used are:

- **Pulsed splitless injection:** With pulsed splitless injection, the flow through the inlet and column is increased during the injection process. This increased flow sweeps analytes out of the inlet much more rapidly than normal splitless, reducing exposure of the analytes to the high temperature of the inlet. This reduces breakdown for active pesticides.
- **RTL:** RTL is an Agilent feature where a locking compound, in this case chlorpyrifos-methyl, is run on the system, and software determines the required column flow rate to get precisely the same RT as that in spectral libraries collected under the locked conditions. This feature results in nearly identical RTs for pesticides across multiple instruments and platforms, making data analysis and method maintenance much easier. Precise RTs make a useful filter in the screening process.
- **Spectral deconvolution:** The spectral deconvolution features in MassHunter Quantitative 10 Unknowns Analysis provide an automated means of quickly identifying compounds in high-matrix samples using library match score and, if available, precise RT matching.

Figure 1 shows the system configuration used in this work.

Table 1 lists the instrument operating parameters. Pulsed splitless injections were used to maximize the transfer of pesticides, especially the active ones, into the column. Initially, problems with analyte peak shapes were encountered due to the use of acetonitrile as the injection solvent. Acetonitrile is known to be troublesome with splitless injections into the seminonpolar columns used. The Agilent single taper Ultra Inert splitless inlet liner (part number 5190-2293) (top of Figure 2) is widely used for splitless injection and works well with most common GC solvents. With acetonitrile, however, pulsed splitless injections produced multiple peaks for each analyte. The Agilent Ultra Inert universal low pressure drop inlet liner (part number 5190-2295) (bottom of Figure 2), combined with adjusting the initial oven temperature and hold, was found to eliminate the problem, and was used for all subsequent analyses. Note that this problem is volume-dependent, and that injections here were limited to 1.0 µL.

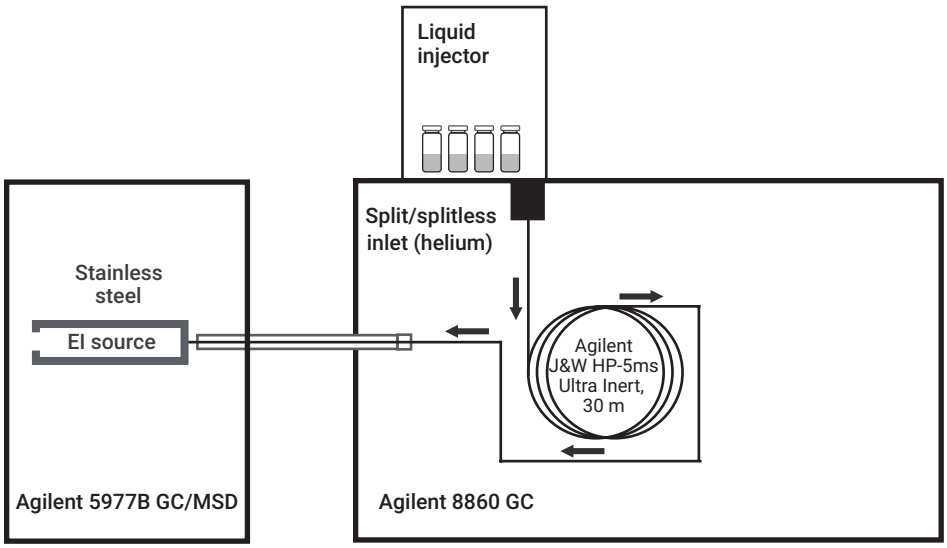

Figure 1. Configuration of the Agilent 8860 GC and Agilent 5977B GC/MSD systems.

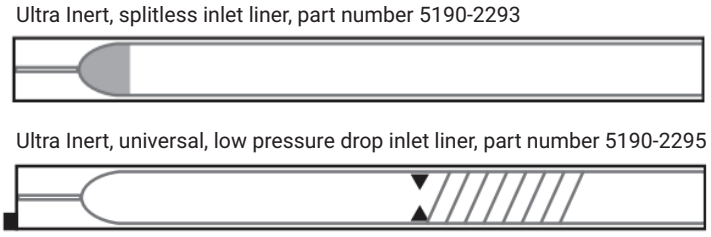

Figure 2. Agilent inlet liners evaluated for pulsed splitless injection.

Sample preparation

Sixteen different packages of organic and nonorganic strawberries were purchased at local retail stores as well as at farmer's markets. Strawberries were cut into small pieces, frozen, and blended under liquid nitrogen (organic samples were blended first). A QuEChERS sample preparation was used as follows. Ten grams of each sample were weighed into a 50 mL centrifuge tube. Two ceramic homogenizers were added to each centrifuge tube, followed by the addition of 10 mL of acetonitrile (HPLC grade) to each tube. Samples were mechanically shaken for three minutes at 1,500 strokes/min. An EN method 15662 QuEChERS extraction salt packet (part number 5982-6650) was added to each centrifuge tube. Samples were mechanically shaken for three minutes at 1,500 strokes/min then centrifuged for five minutes at 5,000 rpm. A 6 mL aliquot of the extract was transferred to a QuEChERS Dispersive SPE 15 mL tube (general fruits and vegetables, part number 5982-5056). Samples were vortexed for three minutes at 1,500 strokes/min, then centrifuged for five minutes at 5,000 rpm. The sample extracts were then transferred to labeled autosampler vials for analysis.

Table 1. GC/MS conditions for pesticide screening.

GC	
Agilent 8860 GC system with auto-injector and tray	
Inlet	
	Split/splitless inlet
Mode	Pulsed splitless
Injection Pulse Pressure	50 psi until 0.75 minutes
Purge Flow to Split Vent	50 mL/min at 0.7 minutes
Injection Volume	1.0 µL
Inlet Temperature	280 °C
Carrier Gas	Helium
Inlet Liner	Agilent low pressure-drop (LPD) with glass wool (p/n 5190-2295)
Oven	
Initial Oven Temperature	80 °C
Initial Oven Hold	1.5 minutes
Ramp Rate 1	40 °C/min
Final Temperature 1	120 °C
Final Hold 1	0 minutes
Ramp Rate 2	5 °C/min
Final Temperature 2	310 °C
Final Hold 2	10 minutes
Total Run Time	50.5 minutes
Post Run Time	0 minutes
Equilibration Time	0.25 minutes

Column	
Type	Agilent J&W HP-5ms Ultra Inert (p/n 19091S-433UI)
Length	30 m
Diameter	0.25 mm
Film Thickness	0.25 µm
Control Mode	Constant flow
Flow	1.374 mL/min
Inlet Connection	Split/splitless
Outlet Connection	MSD
MSD	
Model	Agilent 5977B GC/MSD
Source	Stainless steel
Vacuum Pump	Performance turbo
Tune File	Atune.U
Mode	Scan
Scan Range	45 to 550 amu
Solvent delay	4 minutes
EM voltage Gain mode	1.0
TID	On
Quad Temperature	150 °C
Source Temperature	280 °C
Transfer Line Temperature	280 °C

Results and discussion

Screening scan data: RTL pesticide library

Figure 3 shows the scan total ion chromatogram (TIC) of the sample 21 extract. Although the QuEChERS extraction process is effective at recovering pesticides from the strawberries, it still brings over many matrix compounds, as shown in Figure 3.

The scan file for extract 21 was then run through MassHunter Unknowns Analysis with the deconvoluted components searched against the RTL pesticide library. Figure 4 shows the report generated. The report can be sorted by any of the columns, and is shown sorted by decreasing LMS. Using the fifth entry, fenhexamid, as an example, confidence in it being present is high because it has a high LMS (91.9), and its RT falls within 0.0619 minutes of that in the RTL library. The report shows nine pesticides with LMS values greater than 65 and close RT matches. Figure 5 shows a portion of the TIC of extract 21 with the identified components in green and the fenhexamid component in red. The TIC shows significant amounts of matrix interferences coeluting with the fenhexamid.

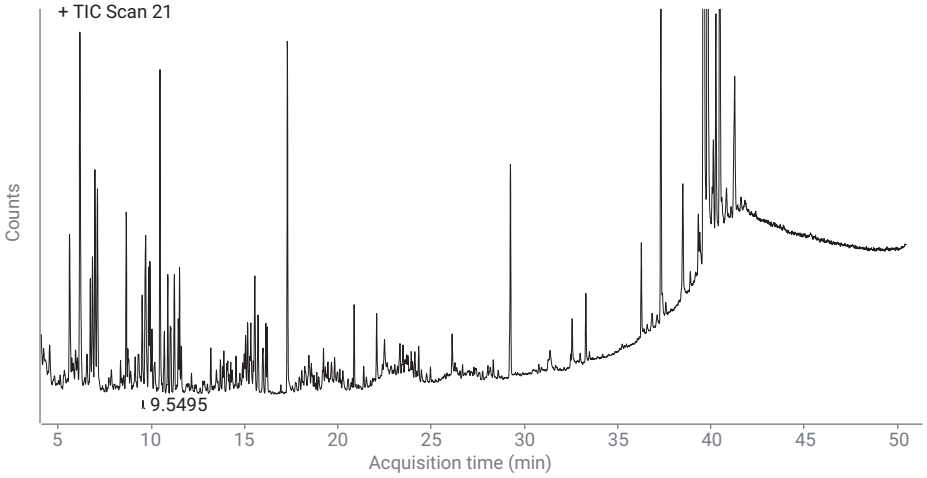

Figure 3. TIC of the extract of sample number 21.

Components

Component RT	Compound Name	Match Factor	Delta RT	Formula	Base Peak Area	
9.9284	Tetrahydrophthalimide, cis-1,2,3,6-	96.9	0.0756	C8H9NO2	71101.6	Pesticides found
20.8760	Cyprodinil	96.7	0.0270	C14H15N3	61475.7	
23.3407	Fludioxonil	96.6	0.0513	C12H6F2N2O2	15070.2	
16.1407	Pyrimethanil	94.2	0.0153	C12H13N3	66782.7	
26.1321	Fenhexamid	91.9	0.0619	C14H17Cl2NO2	35885.2	
21.3895	Captan	89.1	0.0395	C9H8Cl3NO2S	13758.1	
19.3621	Di-n-butylphthalate	86.4	0.0199	C16H22O4	6234.7	
12.3959	Flonicamid	85.0	0.0131	C9H6F3N3O	5788.6	←
8.2805	Novaluron	84.4	0.0425	C17H9ClF8N2O4	2973.1	←
20.7134	Sulfur (S8)	80.5	-0.1854	S8	4940.3	
10.4643	Cashmeran	75.9	0.0377	C14H22O	249203.7	
17.5668	Diisobutyl phthalate	73.5	0.0152	C16H22O4	2909.3	
28.2554	Bifenazate	70.8	0.0706	C17H20N2O3	949.3	←
12.8967	Benzophenone	69.4	0.0223	C13H10O	4619.0	
5.0282	2,4-Dimethylphenol	67.3	-0.0732	C8H10O	3014.1	
12.1536	Diethyl phthalate	65.3	0.0194	C12H14O4	5618.9	

Figure 4. Search results for sample 21 against RTL pesticide library.

Figure 6 shows the information displayed when inspecting a hit, in this case fenhexamid, in MassHunter Unknowns Analysis. Figure 6A overlays the EICs of the ions the software has identified as being part of the spectrum. The overlay is inspected to see if the EICs all have similar shape and RT, as they do here. The spectrum in Figure 6B is the average of the raw spectra over the component profile of the peak. Its purpose is to show the degree of interfering ions from coeluting compounds. The spectrum shows the presence of interferences, as suggested by the TIC in Figure 5.

Figure 6C shows the deconvoluted spectrum of the component found at the RT of fenhexamid compared to the inverted library reference spectrum. The deconvolution process had removed the interfering ions, producing a high-quality LMS of 91.9. Taken with the precise time match, there is high confidence in fenhexamid being present in sample 21.

The inspection process was repeated for all the hits found in MassHunter Unknowns Analysis to generate a list of compounds of interest for quantitation.

The decision as to what compounds to add to the list depends on several factors such as LMS, RT match, degree of concern for a specific compound, and so forth.

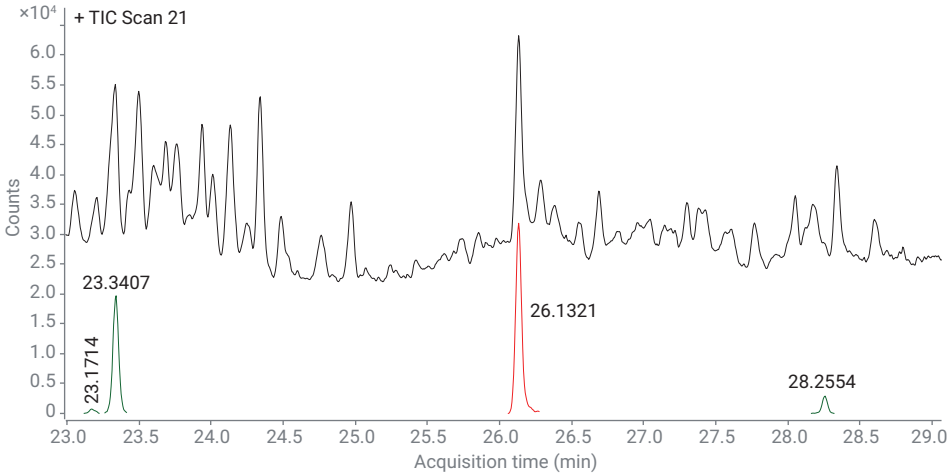

Figure 5. TIC of the extract of sample number 21 (black trace) identified components (green trace) and fenhexamid component (red trace).

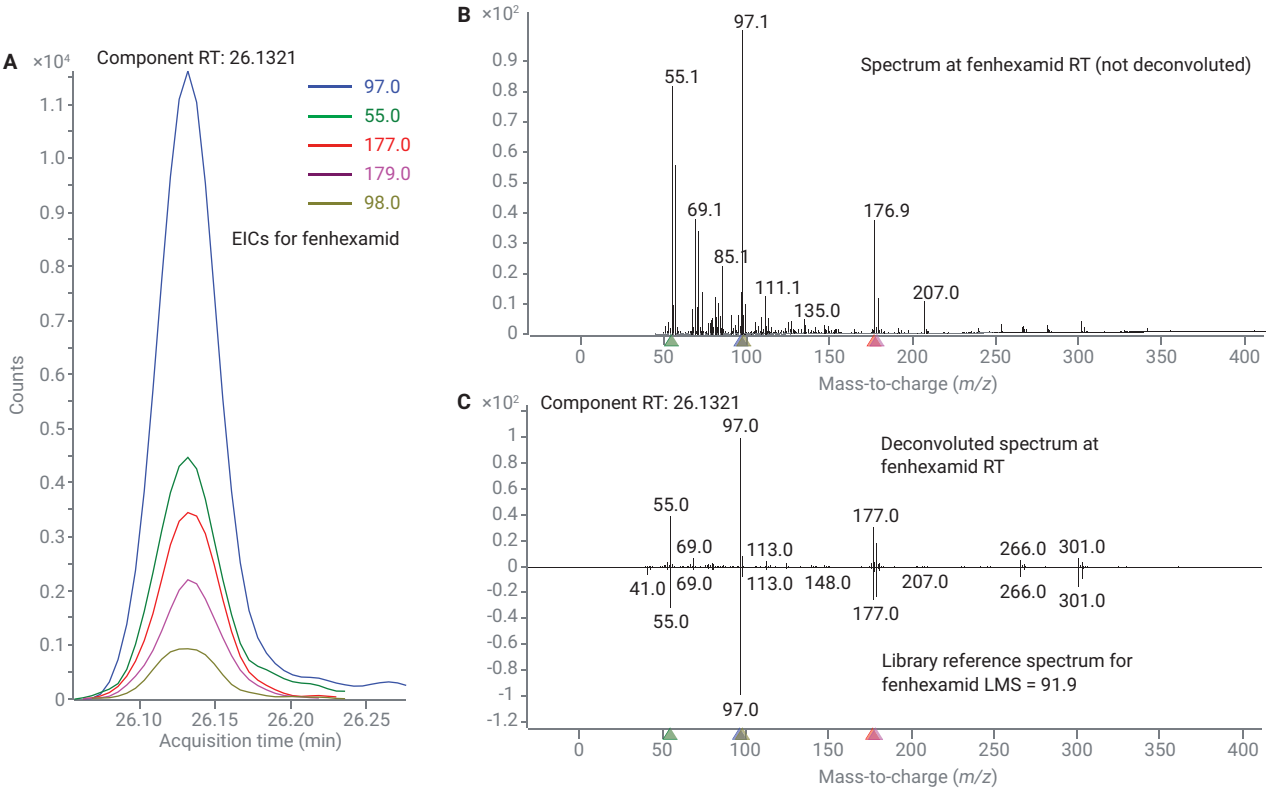

Figure 6. Identification of fenhexamid in extract 21 with MassHunter Unknowns Analysis.

The Base Peak Area item is also useful as an indication of the relative size of the response for the listed hit. Typically, compounds with LMS scores less than 65 would be ignored unless the compound is of high concern.

To illustrate the inspection of a hit with a marginal LMS, fenhexamid appears present in sample extract 19 at a level substantially lower than in sample 21. Figure 7 shows the spectral information displayed in MassHunter Unknowns Analysis for the hit. Based only on spectral match, this hit would probably be rejected. However, since three of the principle ions are present in approximately the right ratios, and the RT is within 0.066 minutes of that in the RTL library, the hit may be worthy of adding to the list of compounds to be quantified.

Screening scan data: NIST 17 library

The >1,000 compound RTL library is convenient for screening because the RT matches are very good, and the number of hits to be inspected is limited. However, there are cases when a much broader screen may be desired, such as when a new supplier is being evaluated.

MassHunter Unknowns Analysis can also be used to search the deconvoluted components against the NIST 17 library, which contains over 260,000 spectra. NIST 17 contains RIs experimentally determined on semistandard nonpolar columns of the type used here for many of the entries. An alkane RI calibration mix is run with the RTL pesticide method, and is used to create an RI calibration file. MassHunter Unknowns Analysis then searches the deconvoluted spectra through NIST 17 and lists the LMS and RI values for hits as well as the NIST RI values, if available. This is a very powerful tool, but because it searches all matrix components, this can lead to a very large list of hits to be inspected. For example, the screens of the strawberry extracts often produced over 400 hits with LMS values >65.

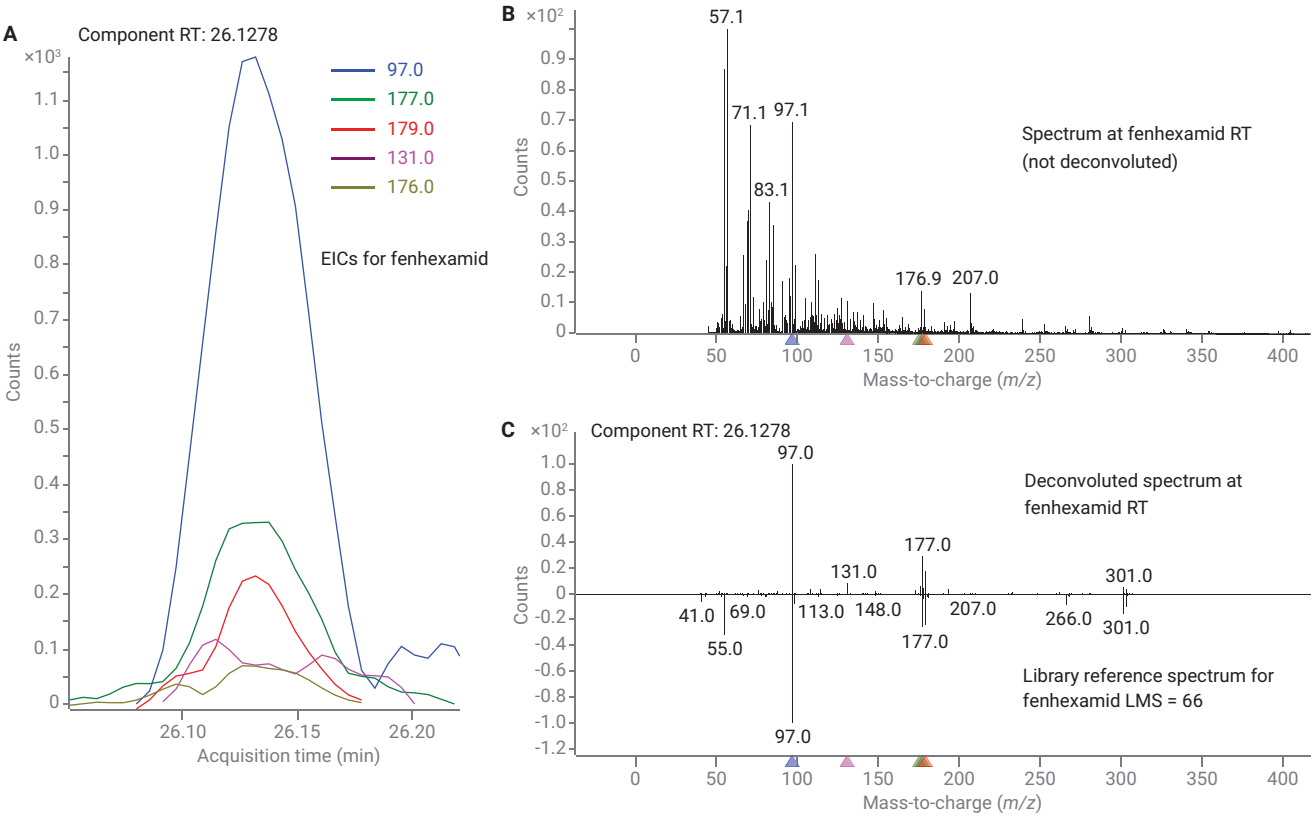

Figure 7. Fenhexamid component in extract 19. A lower amount present results in a lower match score.

Figure 8 shows a portion of the screen results from NIST 17 for extract 21. The component RI was calculated using the hydrocarbon RI calibration. The Library RI is taken from the NIST entry, and is either the experimental RI for the semistandard nonpolar phase, if available, or a theoretical value calculated from molecular parameters. Note that the latter is of limited value, as the errors in the predicted RI are often quite large.

In reviewing the NIST 17 results, consideration is given to the LMS and delta RI values. If the LMS is high, the delta RI is a small percentage of the RI, and the NIST RI is of the experimentally determined type, then there is solid evidence that the compound is present.

The NIST 17 screen can serve multiple purposes:

- Confirming identifications of compounds found with the RTL pesticide library screen
- Finding alternative identifications for RTL screen hits with questionable LMS values
- Identifying chemicals not in the RTL screen that may be of concern

In Figure 8, fenhexamid is found with a high LMS value (93.7) but a rather large delta RI value (of the estimated type) of 159 compared to an RI of 2,349. In this case, with such a high LMS and with the uncertainty in estimated library RIs, fenhexamid's presence would be considered as likely. It was already confirmed present with the RTL pesticide library screen. The NIST 17 search results also show cyprodinil, pyrimethanil, and fludioxonil as having very high LMS values and very low delta RI values of the experimental type, confirming the identification of these compounds found with the RTL pesticide library screen.

While reviewing the NIST 17 search results for extract 19, a hit identified as sarin was listed with an LMS of 78.1. This value of LMS is high enough to warrant further inspection by the data reviewer. As sarin is a chemical warfare agent, it would be of the highest concern if it were present in food.

Components

Component RT	Compound Name	Match Factor	CAS#	Formula	Component RI	Library RI	Delta RI	Base Peak Area	
10.4643	2,4-Di-tert-butylphenol	99.0	96-76-4	C14H22O	1512	1519	7	249203.7	
8.6566	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	98.9	126-86-3	C14H26O2	1414	1407	-7	89233.1	
37.3234	Vitamin E	98.9	59-02-9	C29H50O2	3137	3136	-1	328658.4	
29.2587	Bis(2-ethylhexyl) phthalate	98.7	117-81-7	C24H38O4	2548	2529	-19	169492.9	
39.6297	.gamma.-Sitosterol	98.6	83-47-6	C29H50O	3321	3321	0	425291.4	
20.8760	Cyprodinil	98.2	121552-61-2	C14H15N3	2045	2037	-8	61471.9	Experimental RI in NIST 17
11.5135	1,6,10-Dodecatrien-3-ol, 3,7,11-trim...	98.2	40716-66-3	C15H26O	1565	1564	-1	38323.6	
6.1720	Benzene, 1,3-bis(1,1-dimethylethyl)-	98.1	1014-60-4	C14H22	1256	1249	-7	482927.1	
39.8264	Stigmasta-5,24(28)-dien-3-ol, (3.bet...	98.1	481-14-1	C29H48O	3337	3343	6	350648.9	
9.9284	1,2,3,6-Tetrahydrophthalimide	98.1	85-40-5	C8H9NO2	1483	1470	-13	71101.6	
4.2235	Benzaldehyde, 4-methyl-	97.1	104-87-0	C8H8O	1085	1079	-6	35691.6	
16.1407	Pyrimethanil	96.9	53112-28-0	C12H13N3	1797	1793	-4	66788.4	Experimental RI in NIST 17
23.3407	Fludioxonil	96.3	131341-86-1	C12H6F2N2O2	2183	2169	-14	15070.2	Experimental RI in NIST 17
36.2745	.gamma.-Tocopherol	95.7	7616-22-0	C28H48O2	3054	3074	20	70828.8	
17.2976	Acetic acid, 10,11-dihydroxy-3,7,11...	94.6	1000194-28-5	C17H30O4	1856	2103	247	80191.2	
22.0848	Phytol	93.9	150-86-7	C20H40O	2111	2114	3	34328.4	
5.6150	Benzofuran, 2,3-dihydro-	93.9	496-16-2	C8H8O	1214	1224	10	159798.8	
4.4648	Benzene, 1-isocyano-3-methyl-	93.9	20600-54-8	C8H7N	1110			17760.3	
26.1321	Fenhexamide	93.7	126833-17-8	C14H17Cl2NO2	2349	2508	159	35885.2	Estimated RI in NIST 17
9.5096	Dodecane, 4,6-dimethyl-	93.7	61141-72-8	C14H30	1461	1325	-136	47311.5	
33.2985	Squalene	93.3	111-02-4	C30H50	2828	2832	4	41092.9	

Figure 8. Partial search results for sample 21 against the NIST 17 library.

Components

Component RT	Compound Name	Match Factor	CAS#	Formula	Component RI	Library RI	Delta RI	Base Peak Area
8.0918	4-Chlorobutyric acid, 4-isopropylphenyl ester	58.2	100035...	C13H17Cl...	1382	1813	431	518.2
8.1053	Octanoic acid, 4-isopropylphenyl ester	65.8	100033...	C17H26O2	1382	1905	523	531.8
8.2121	5t-Butyl-4-methylimidazole	56.6	146979-...	C8H14N2	1389	1140	-249	1145.0
8.3395	Sarin	78.1	107-44-8	C4H10FO2P	1397	820	-577	3723.8
8.3507	Undecane, 4,7-dimethyl-	79.2	17301-3...	C13H28	1397	1185	-212	19703.0
8.3507	Tetradecane	92.4	629-59-4	C14H30	1397	1400	3	19703.0
8.3789	Dimethyl-(allyl)-silyloxybenzene	56.9	66998-6...	C11H16OSi	1399	1232	-167	279.8
8.4180	3,5-Dibutoxy-1,1,1,7,7,7-hexamethyl-3,5-bis...	61.3	72439-8...	C20H54O...	1401	2001	600	1302.9

Figure 9. Partial search results for extract 19 against the NIST 17 library.

Figure 10 shows the information as displayed in MassHunter Unknowns Analysis.

The library spectrum of sarin only has two significant ions, and their masses are rather common. Those two ions dominate the LMS calculation, resulting in the 78.1 score. There is also a very large discrepancy between the measured and library (experimental) RI values. The presence of sarin can ultimately be dismissed based on the RI value and relatively poor spectral selectivity.

The extracts of the strawberry samples were also used in a separate experiment[3] that quantified the pesticides found here in the screening process. By comparing the screening results with the quantitation values, an estimate of the amount of pesticide required for identification by the screening process was made.

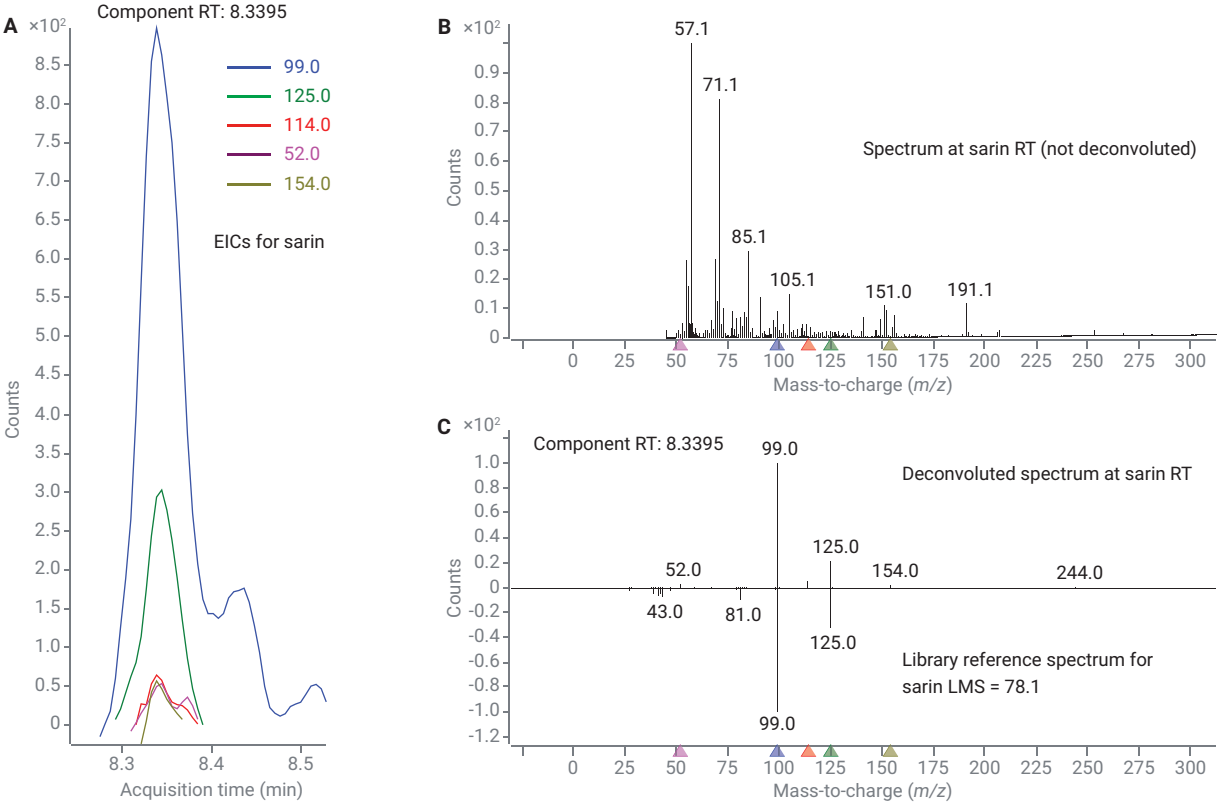

Figure 10. Examination of a deconvoluted spectrum identified by LMS in NIST 17 as sarin in extract 19.

Table 2 contains the pesticides identified in the strawberry extracts, the tolerances for the maximum concentration of a pesticide residue in strawberries established by the US EPA,[4,5] and the estimated amount required for identification by screening. All of the pesticides encountered in the strawberry samples could be identified at or below the allowed tolerance levels.

Conclusion

The Agilent 8860 GC and Agilent 5977B GC/MSD system provided a cost-effective means of identifying pesticides in strawberries. Pulsed splitless injection produces suitably inert sample transfer at the required levels. By first screening sample extracts in scan mode using Agilent MassHunter Unknowns Analysis software, which provides automated deconvolution and library searching, pesticides or other chemicals of concern can be found.

The use of RTL also allows results to be easily compared with those obtained on other instruments and MS types. Any compounds of interest found with this system can be compared to results obtained with GC/MS/MS using the Agilent pesticides and environmental pollutants MRM database. They can also be compared to results obtained with GC/Q-TOF and Agilent MassHunter Quantitative Analysis and an accurate mass Pesticide Personal Compound Database and Library (PCDL). The use of multiple platforms provides a powerful toolset for addressing the needs of food safety.

Table 2. Estimated ppb of pesticides required for identification with this method.

Compound	Tolerance (ppb)	ppb Required to Identify
Azoxystrobin	10.000	600
Bifenazate	1,500	500
Bifenthrin	3,000	100
Captan	20,000	2,000
cis-1,2,3,6-Tetrahydrophthalimide	25,000	500
Cyprodinil	5,000	100
Etoxazole	500	300
Fenhexamid	3,000	300
Flonicamid	1,500	300
Fludioxonil	2,000	100
Malathion	8,000	150
Metalaxyl	10,000	100
Myclobutanil	500	500
Novaluron	500	500
Pyrimethanil	3,000	100
Quinoxyfen	900	100
Tetraconazole	2,500	150
Trifloxystrobin	1,100	150

References

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