

Rapid Rinse and Shoot: Screening Workflow for Pesticides in Fruit by GC/MSD in Under Six Minutes

Authors

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Abstract

The Agilent Intuvo 9000/5977B GC/MSD system enabled a fast screening workflow for residual pesticides present on the surface of fruits. This method used a GC oven with direct heating technology and MS spectral deconvolution. Residual pesticides were rinsed from the tested commodity surface with acetone. The rinsate was collected and injected into the GC/MSD system. The direct heating oven allowed a very high temperature program rate (250 °C/min) to complete the GC/MS analysis in 3.4 minutes. Spectral deconvolution coupled with the library search algorithm and time-filtering using retention indices resulted in rapid and confident identification of residual pesticides present on the fruit. The NIST 17 spectral library, other commercially available libraries, and user created libraries can be used for compound identification. MassHunter Unknowns Analysis software provides capabilities to create custom reports. The entire analysis from sample collection to reporting took under 6 minutes. The combination of the Intuvo Guard Chip and column backflushing yielded longer maintenance-free uptime. This approach is particularly useful for prioritizing samples for more in-depth analysis.

Introduction

Trace-level pesticide and environmental pollutants in the food supply continue to be a worldwide concern. These concerns are driving the demand for more rapid and reliable methods of analysis. The challenge is to find technologies that can search for hundreds of pesticides, PAHs, and other targets with simple sample preparation and a quick turnaround time.

The targeted analytical methods commonly used in food safety analysis focus on a finite list of compounds. This approach typically uses gas and liquid chromatography coupled with triple quadrupole mass spectrometry (GC/TQ and LC/TQ).¹ While these techniques provide outstanding sensitivity, the limitation is a rather restricted scope of targets, even if the method includes several hundreds of pesticides. High-resolution mass spectrometry (HRMS) techniques coupled with GC and LC would enable comprehensive sample screening.² However, this approach typically requires high-resolution databases to analyze data against them. Such databases often include only a limited number of compounds, and creating an expert-curated library is a labor-intensive process. In addition, HRMS requires rather sophisticated and expensive instrumentation. An alternative screening approach uses GC coupled with a single quadrupole mass spectrometer (GC/MSD) operated in a full scan mode.^{3,4} This screening technique is used in the Agilent QuickProbe GC/MS System. The technique enables direct analysis in real time of powders, tablets, and liquids with minimal to no sample preparation, in under 1 minute. The QuickProbe is available on 5977B GC/MSDs with 8890 GC or 7890B GC systems. The system enables rapid heating and separation with the QuickProbe inlet and short separation column.⁵ To increase the confidence of identification with

GC/MSD, mass spectral deconvolution and time-filtering is used. Time-filtering can be performed based on the retention times (RT) of the identified targets by comparing them to the library RT values. The disadvantage of RT-filtered search is its dependence on the flow path, column flow, and oven ramp rate. The use of linear retention indices (RI) makes the screening strategy independent of these chromatographic conditions. Also, commercial libraries commonly include RIs rather than RTs. When time-filtering is performed with only RIs available, the RI values of the identified targets and the RI values from the library are recalculated to RTs using a RI-to-RT calibration. The calculated RTs for the identified compounds are compared to the RTs calculated for the library entries. Only the targets with the calculated RTs within the time-filtering range of the RTs calculated for the library entries are shown in the report. The conversion of the RIs to RTs is performed by MassHunter Unknowns Analysis software for user convenience. Both RIs and RTs values can be shown in the report.

The screening approach described in this application note uses a GC/MSD system for a rapid (3.4 minutes) analysis. Fruit rinsates, compound identification based on deconvolution mass spectral search, and time-filtering using RIs provide enhanced confidence in identification. Acetone rinsates of fruit provided a qualitative estimate of residual foliar applied pesticides. The advantage of this sample collection technique is a high pesticide-to-matrix ratio compared to sample preparation techniques that involve sample grinding and extraction. This approach can be successfully implemented for initial commodity triage. Any fruit for which the rinsate is found to contain compounds of concern, can be further subjected to a comprehensive and quantitative sample preparation, for example, QuEChERS, followed by targeted quantitative analysis

performed with GC/MSD operated in selected ion monitoring (SIM) mode or GC/TQ in multiple reaction monitoring (MRM) mode.

The entire triage workflow was performed in under 6 minutes, including fruit rinsing, GC/MSD analysis, data processing, and report generation. The unprecedented speed of GC/MSD screening performed in 3.4 minutes was achieved using the Agilent Intuvo 9000 GC. This system provides oven ramping at a rate of 250 °C/min without requiring special electrical service (V or A) at the bench. In addition, its small footprint makes it amenable to analysis outside of conventional laboratories, such as receiving docks and distribution centers.

The utility of this approach was demonstrated by analysis of five types of fruits and berries. The fruit samples were strawberries, bananas, lemons, cherries, and peaches. These fruits were purchased in a local grocery store and a farm around Wilmington, DE and subjected to analysis with the method.

Experimental

The system used here was configured to enable the shortest cycle time, avoid carryover, and maximize throughput. The important techniques employed were:

- An oven ramp rate of 250 °C/min, achieved with the Intuvo 9000 GC, enabling a 3.4-minute run time. Note that the Intuvo 9000 GC does not require special electrical service (V or A) at the bench, and operates with a standard 120 V power outlet.
- Midcolumn backflushing extends the life of the columns and the Guard Chip. Backflushing is a technique in which the carrier gas flow is reversed after the last analyte has exited the column. After the MS data are collected, the oven is held at the final temperature in postrun mode, and the carrier gas flow through the

first column and the Guard Chip is reversed. This reversed flow carries any high boilers that were in the column and the Guard Chip at the end of data collection out into the split vent trap. For this configuration, the backflushing time was 1 minute. The Intuvo 9000 GC enables self-configuration when setting up backflush and columns, which are equipped with the column information keys, that significantly simplifies method setup.

- The Intuvo PSD module is an Intuvo 9000 pneumatics module optimized for backflushing applications. During backflushing, it significantly reduces the flow of helium used compared to previous configurations. The PSD allows simpler setup of backflush.
- The Intuvo 9000 MMI Guard Chip prevents high-boiling matrix compounds from contaminating the head of the column. This greatly reduces the need for column trimming and increases the column's lifetime. Changing the Guard Chip instead of trimming the column also preserves the retention times, thus reducing the need for RI recalibration.
- Multimode inlet (MMI) enables temperature-programming. The inlet temperature was increased in postrun to minimize carryover caused by residual sample matrix accumulated in the inlet.
- The spectral deconvolution feature in Agilent MassHunter Quant 10.1 Unknowns Analysis (MH UA) provides an automated means of quickly identifying compounds even in high-matrix samples in the presence of coeluting compounds using library match score (LMS).
- Time filtering using RIs increased compound identification accuracy. MH UA recalculates RI values

observed for the component in the sample and RI values available in the library to RTs using RI calibration. The difference between the calculated RTs for the observed component and the library entry is listed in the report. Unlike RT, RI is independent of the flow path and is applicable to a custom column configuration.

- MH UA customizable reporting for presentation of the results.
- SIM mode can be used for

enhancing analysis sensitivity when monitoring only a limited number of target compounds.

Figures 1A and 1B show the system configuration and the Intuvo 9000/5977B GC/MSD system used in this work. Figure 1C shows the 7650A automatic liquid sampler with a 50-vial capacity that was used in this application, while Figure 1B features a standard 7693A autoinjector.

The instrument operating parameters are

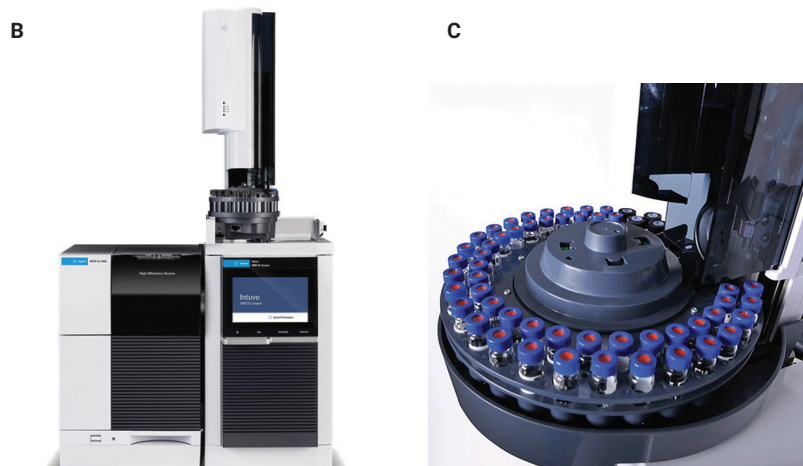
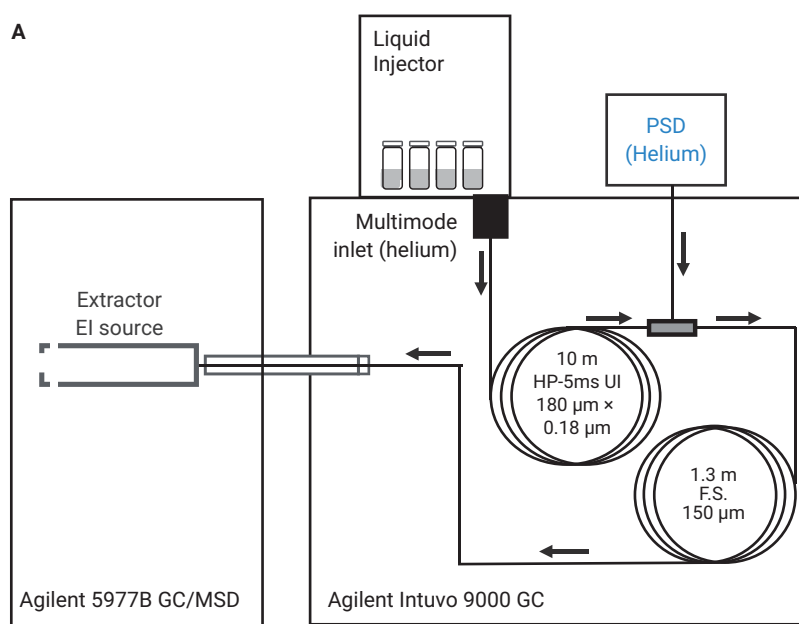


Figure 1. (A) System configuration. (B) The Agilent Intuvo 9000/5977B GC/MSD system with a standard 7693A auto injector. (C) The Agilent 7650A automatic liquid sampler with a 50-vial capacity used in this work.

listed in Table 1. Custom GC columns were used in this work. (A request for ordering Intuvo GC custom columns can be placed via an [online form](#). The first column was a 10 m × 0.18 mm, 0.18 µm Agilent HP-5ms UI that provided

sufficient chromatographic resolution while maintaining a short run time. The second column was a 1.3 m × 0.15 mm uncoated fused silica that allowed midcolumn backflushing by acting as a column flow restrictor to the mass

spectrometer. To accommodate the flows used with backflushing, the performance turbo vacuum pump is required.

Table 1. GC/MS conditions.

| GC | |
|--|---|
| Agilent Intuvo 9000 with 7650A Automatic Liquid Sampler | |
| Inlet | Multimode Inlet (MMI) |
| Mode | Split |
| Split Ratio | 15:1 |
| Septum Purge Flow | 3 mL/min |
| Injection Volume | 1.0 µL |
| Injection Type | Standard |
| L1 Airgap | 0.2 µL |
| Gas Saver | On at 20 mL/min after 0.7 min |
| Inlet Temperature | 280 °C |
| Post Run Inlet Temperature | 350 °C |
| Post Run Total Flow | 50 mL/min |
| Carrier Gas | Helium |
| Inlet Liner | Agilent universal low pressure drop liner, with glass wool (p/n 5190-2295) |
| Oven | |
| Gradient | 40 °C, hold 0.2 min 250 °C/min to 320 °C, hold 2.08 min |
| Total Run Time | 3.4 min |
| Post Run Time | 1 min |
| Equilibration Time | 1 min |
| Column 1 | |
| Type | Agilent HP-5MS UI, 10 m × 0.18 mm, 0.18 µm (custom Intuvo column) |
| Control Mode | Constant Flow |
| Flow | 0.75 mL/min |
| Inlet Connection | Multimode Inlet (MMI) |
| Outlet Connection | PSD (PUU) |
| PSD Purge Flow | 5 mL/min |
| Post Run Flow (Backflushing) | -4.73 mL/min |
| Column 2 | |
| Type | Agilent Ultra Inert uncoated fused silica, 1.3 m × 0.15 mm (custom Intuvo column) |
| Control Mode | Constant Flow |
| Flow | 1.25 mL/min |
| Inlet Connection | PSD (PUU) |
| Outlet Connection | MSD |
| Post Run Flow (Backflushing) | 16.15 mL/min |
| Intuvo 9000 GC Components | |
| Guard Chip | Intuvo MMI Guard Chip (p/n G4587-60665) |
| Track Oven | On |
| Bus Temperature | 320 °C |

| MSD | |
|--------------------------------|---|
| Model | Agilent 5977B |
| Source | Inert Extractor Source with a 3 mm lens |
| Vacuum Pump | Performance Turbo |
| Tune File | ATUNE.U |
| Mode | Scan |
| Scan Range | 50 to 550 <i>m/z</i> |
| Scan Rate | N = 1 |
| Solvent Delay | 0.97 min |
| EM Voltage Gain Mode | 1 |
| Quad Temperature (MS1 and MS2) | 150 °C |
| Source Temperature | 300 °C |
| Trace Ion Detection | On |

Sample preparation

Five varieties of fruits and berries not labeled as organic, including strawberries, bananas, lemons, cherries, and peaches were purchased at a local retail store and a farm in Wilmington, DE area. The tested fruit was placed into a glass funnel and rinsed with acetone ($\geq 99.9\%$ (dried basis), GC2 for gas chromatography, for pesticide residue analysis, Honeywell Burdick & Jackson, Muskegon, MI) using a 500 mL PTFE squeeze bottle, as shown in Figure 2. With bananas, lemons and peaches, one piece of fruit was placed into the funnel. With strawberries and cherries, several berries were placed in the funnel to fill up 2/3 of the funnel volume. The rinsate was collected into a 4 mL amber vial until the vial was full. The funnel was rinsed on the inside with acetone between the samples.



Figure 2. Simple sample preparation to accompany fast chromatography for quick screening.

RI calibration with *n*-alkanes

It is common in chromatography that RT is a key parameter to aid analyte identification. However, RT depends on the GC flow path, column stationary phase and dimensions, as well chromatographic conditions. Alternatively, relative RT can be used for analyte identification. Originally, relative RTs for isothermal GC separations known as Kovats Indices were used. Later, the calculations of relative retention were adapted to apply to temperature-programmed GC separations and these values became known as Linear Retention Indices (LRI or RI). RI works well for identifying compounds if the oven temperature program is not complex and the stationary phase type is the same as that used to collect the reference library RIs. Application of RI requires that an *n*-alkane mixture is run that covers the elution range of the analytes of interest. Some practitioners use straight chain methyl- or ethyl-esters. In this work, the RI of the latest eluting pesticide in the pesticide library was under 3,400, suggesting that RI calibration needed to be performed up to C_{34} linear *n*-alkane. A detailed workflow describing how to create RI calibration with MassHunter Quantitative Analysis is described in the technical brief.⁶ In this work, RI calibration was performed over the range of linear *n*-alkanes C_9 to C_{34} with a custom *n*-alkane standard (Ultra Scientific, Agilent Technologies Inc., Rhode Island, USA), containing every linear *n*-alkane between C_8 and C_{40} (Figure 3A). The concentration of several alkanes, including C_{13} , C_{18} , C_{22} , C_{28} , and C_{31} was twice that of other alkanes, simplifying their identification.

Backflushing

A postrun backflush was initiated at 3.4 minutes after linear *n*-alkane C_{34} eluted. There was no need to continue the chromatographic run past 3.4 minutes as no pesticides were expected to elute beyond this time. However, heavier matrix components may still be on the GC column by the end of the analytical run. To eliminate carryover and the appearance of ghost peaks in subsequent runs, postrun backflush was initiated at 3.4 minutes by raising the pressure on PSD at the mid-point, hence, reversing the flow through the first column and increasing the flow through the second column. The reversed flow carries any high-boiling compounds that were in the column and the Guard Chip at the end of data collection out into the split vent trap.

Figure 3A shows the chromatogram of *n*-alkane mixture C_8 to C_{40} , which was terminated after C_{34} eluted and followed by a 1-minute postrun backflush. The subsequent blank run is shown in Figure 3B. Note that the C_{35} to C_{40} peaks were eliminated by backflushing and therefore did not appear as carryover peaks.

Figure 3C shows the chromatogram of *n*-alkane mixture C_8 to C_{40} , which was terminated after C_{34} eluted with no backflushing. The subsequent blank run showed the undesired peaks from carryover (Figure 3D). This illustrates that backflushing for 1 minute can replace a time-consuming column bakeout at the end of the run, extending column lifetime. In addition, removing heavy species from the first column and the Guard Chip also extends their lifetime.

In Figure 3, later eluting alkanes tail due to lower transfer line and source temperatures appropriate for pesticides analysis.

Results and Discussion

Fast pesticide screening of fruit and berries

The scan total ion chromatogram (TIC) of a strawberry acetone rinsate is shown in Figure 4. Although sample preparation via rinsing the surface decreased the amount of matrix introduced to the system compared to the conventional sample preparation techniques that involve sample grinding and extraction, it still brought over some matrix compounds, as shown in the scan TIC in Figure 4.

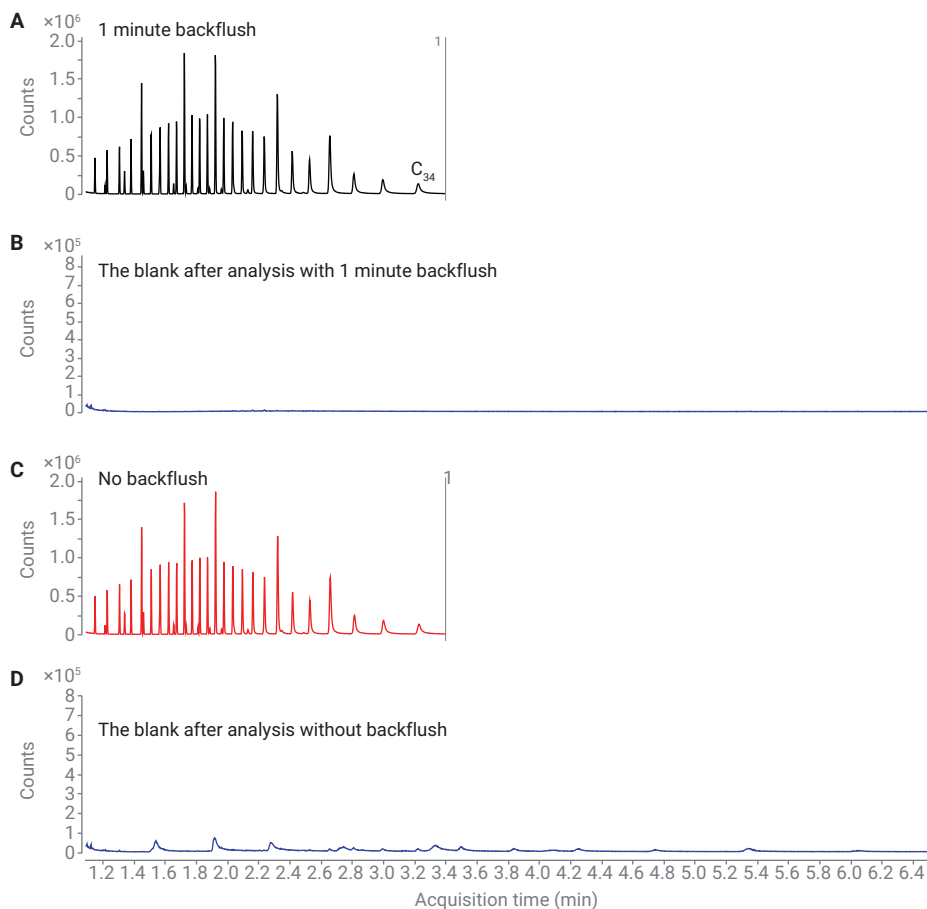


Figure 3. (A) RI Calibration with linear *n*-alkane ladder up to C_{34} in 3.4 minutes; blank runs following a C_8 to C_{40} *n*-alkane analysis aborted after C_{34} elution with a 1-minute backflush (B) and without backflushing (D).

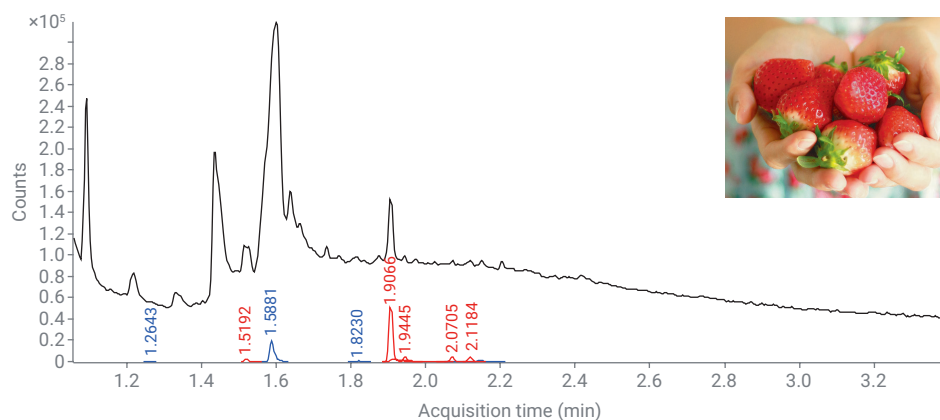


Figure 4. TIC of the strawberry rinsate with acetone.

The scan file for the strawberry rinsate was then run through MH UA with the deconvoluted components searched against a custom pesticide library that included 1,081 entries (spectra and RI) for pesticides and common environmental contaminants. The minimum library match score (LMS) was set to 55 and time filtering was enabled with an RT penalty-free range of ± 12 seconds. As discussed in Experimental, RIs for both the identified components and the library entries were recalculated to the RTs using the RI calibration. Time-filtering with a penalty-free range was performed using the RTs calculated by MH UA automatically. Ten compounds were identified in the strawberry rinsate that met the method criteria. The peaks corresponding to these components are highlighted in blue and red and labeled with their RT in Figure 4. The generated report summarizing all the compounds identified against the library of pesticides and end environmental contaminants is shown in Table 2. The report can be sorted by any of the columns, and is shown sorted by increasing RT. The pesticides identified in the strawberry rinsate sample are labeled in red in Figure 4 and highlighted in blue in Table 2. Among the remaining identified compounds are environmental contaminants such as dicyclohexyl phthalate, possibly from packaging, *cis*-1,2,3,6-tetrahydrophthalimide (a possible metabolite of captan), a fungicide, tolclofos-methyl, and a phenol derivative, 4-methylphenol, of natural origin.

The pesticide library includes the information only on the compound RI but not RT. MH UA uses RI calibration to calculate library RT using the RI and compares the RT of the detected components with the library entries. The filtered hits are displayed in the report. In Table 2, only RT values are shown. If desired, RI values also can be displayed in the report.

As an example, one of the reported pesticides in Table 2, fenhexamid, had a low LMS of 63.6. However, confidence in the identification was improved because of the small delta RT (-0.064 minutes), which is the difference between the

observed RT and that calculated based on the library RI. Additionally, ions present in the deconvoluted spectrum for fenhexamid were relatively unique and matched those from the inverted library reference spectrum (Figure 5, top right). The spectral deconvolution process had removed the interfering ions that appeared in the spectrum before deconvolution (Figure 5, bottom right), producing an LMS of 63.9, which would not have been achieved for the raw spectrum. The alignment of extracted ion chromatograms for characteristic ions is shown on the left in Figure 5.

Table 2. Search results for the strawberry rinsate against the pesticide library.

| Components | | | | | | | |
|--------------|------------|----------|----------------------------------|--------------|-------------------------------------|-------------|----------------|
| Component RT | Library RT | Delta RT | Compound Name | Match Factor | Best Hit | Formula | Component Area |
| 1.2643 | 1.2957 | 0.0314 | 4-Methylphenol | 56.8 | <input checked="" type="checkbox"/> | C7H8O | 125.3 |
| 1.5192 | 1.5094 | -0.0098 | Novaluron | 60.8 | <input checked="" type="checkbox"/> | C17H9ClF... | 1755.9 |
| 1.5881 | 1.5607 | -0.0274 | <i>cis</i> -1,2,3,6-Tetrahydr... | 77.3 | <input checked="" type="checkbox"/> | C8H9NO2 | 18090.7 |
| 1.8230 | 1.7755 | -0.0476 | Tolclofos-methyl | 56.8 | <input checked="" type="checkbox"/> | C9H11Cl2... | 539.5 |
| 1.9066 | 1.8585 | -0.0482 | Captan | 88.3 | <input checked="" type="checkbox"/> | C9H8Cl3N... | 37205.7 |
| 1.9154 | 1.8537 | -0.0617 | Thiabendazole | 63.7 | <input checked="" type="checkbox"/> | C10H7N3S | 6137.1 |
| 1.9445 | 1.9161 | -0.0284 | Fludioxonil | 64.2 | <input checked="" type="checkbox"/> | C12H6F2... | 3764.8 |
| 2.0705 | 2.0062 | -0.0644 | Fenhexamid | 63.6 | <input checked="" type="checkbox"/> | C14H17Cl... | 4593.0 |
| 2.1184 | 2.0849 | -0.0335 | Fluxapyroxad | 63.2 | <input checked="" type="checkbox"/> | C18H12F5... | 3811.7 |
| 2.1462 | 2.1097 | -0.0365 | Dicyclohexyl phthalate | 56.8 | <input checked="" type="checkbox"/> | C20H26O4 | 2573.3 |

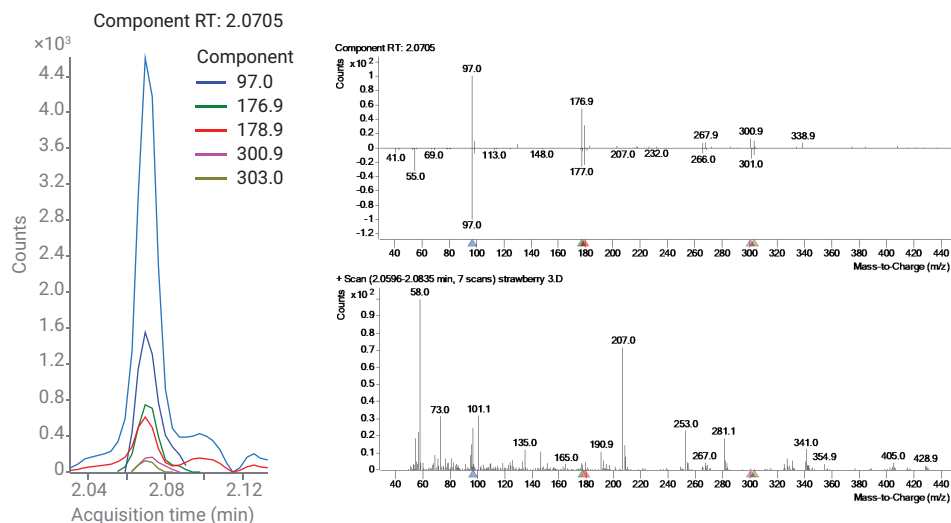


Figure 5. Identification of fenhexamid in the strawberry rinsate with Agilent MassHunter Unknowns Analysis.

The inspection process of the compounds reported in the sample is repeated for all the hits found with MH UA to generate a list of compounds included in the final report. The decision as to what compounds to add to the report depends on several factors such as LMS, RT match, degree of concern for a specific compound, etc. The Base Peak Area item is also useful as an indication of the relative size of the response for the listed hit.

The custom mass spectral library with >1,000 compounds that contains exclusively pesticide and environmental contaminants is convenient for screening because the number of hits to be inspected is limited. However, there are cases when a much broader screen may be desired. MH UA can also be used to search the deconvoluted components against the NIST 17 library, which contains over 260,000 spectra. NIST 17 contains RTs experimentally determined on “semi-standard non-polar” columns of the type used here for many of the entries. MH UA then searches the deconvoluted spectra through NIST 17 and lists the LMS. To increase confidence of identification, time-filtering is performed using the algorithm described above. Briefly, the NIST RT values are automatically recalculated to RTs by MH UA using the RI calibration. The RTs for the hits are compared to the calculated NIST RT values to enable time filtering. Although this is a very powerful tool, it searches all matrix components and can produce a very large list of hits to be inspected. For example, the screen of the strawberry rinsate produced 135 hits with LMS values >55 when time-filtering was applied. Time-filtering limited the number of compounds included in the report from 300 to 135. Reviewing the search results from NIST 17 with over 100 hits is more time-consuming than that from the custom library.

A portion of the screen results from NIST 17 for the strawberry rinsate is shown in Figure 6. The library RT shown in the table is calculated based on the RI value from NIST 17 using RI calibration. The RI value from NIST 17 used for calculating the RT is either the experimental RI for the “semi-standard non-polar” 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 can be quite large. Fludioxonil, a fungicide identified in the strawberry rinsate when searching against the pesticide library, was also identified against NIST 17 with a comparable LMS of 61.3 and a small RT delta of -0.038 minutes.

NIST search is useful for comprehensive screening to uncover unexpected compounds. It takes longer to review because of the large number of naturally occurring compounds from the matrix. Also, some compounds identified against the pesticide library may not be present in NIST 17. For example, fluxapyroxad, which was found in the strawberry rinsate, is not included in NIST 17.

The NIST 17 screen can serve multiple purposes:

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

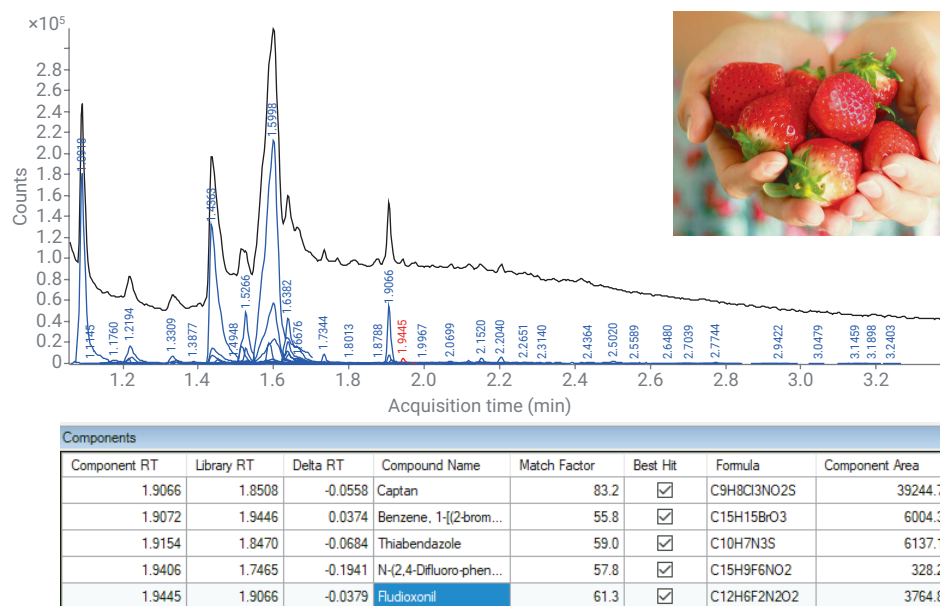
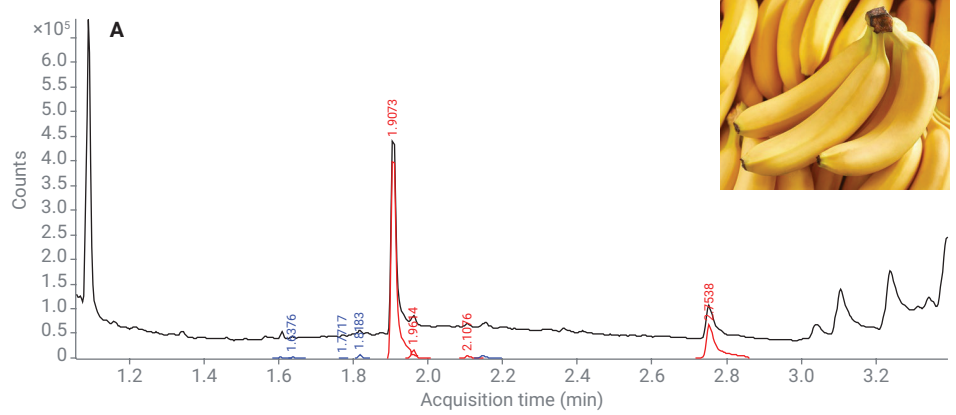
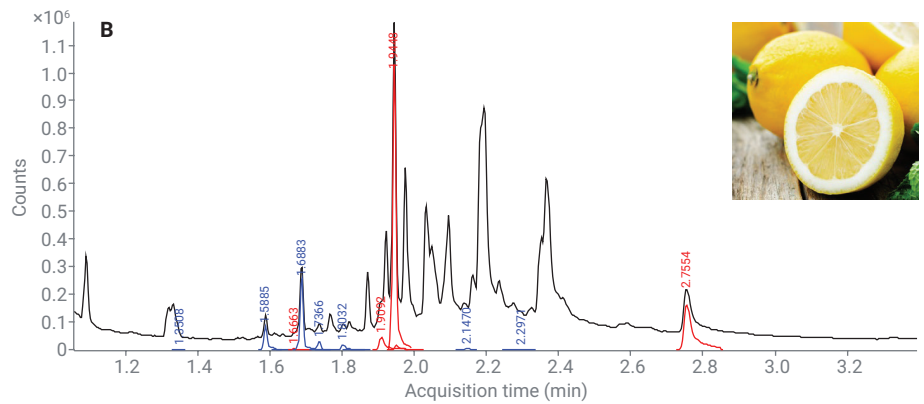


Figure 6. Search results for the strawberry rinsate against NIST 17 spectral library.

Other fruits and berries, including bananas, lemons, cherries, and peaches, were screened for pesticides, and their rinsates were analyzed with MH UA against the pesticide library. The results are shown in Figures 7A to 7D with the pesticides highlighted in the tables in blue. Note that various fruits resulted in different levels of matrix background in the scan TIC. Bananas and cherries showed lower backgrounds while strawberries, lemons, and peaches produced a higher scan TIC. In some cases, base peaks in chromatograms were attributed to pesticides detected in acetone rinsates due to a favorable pesticides-to-matrix ratio, e.g., thiabendazole in bananas, fludioxonil in lemons, and captan in peaches.

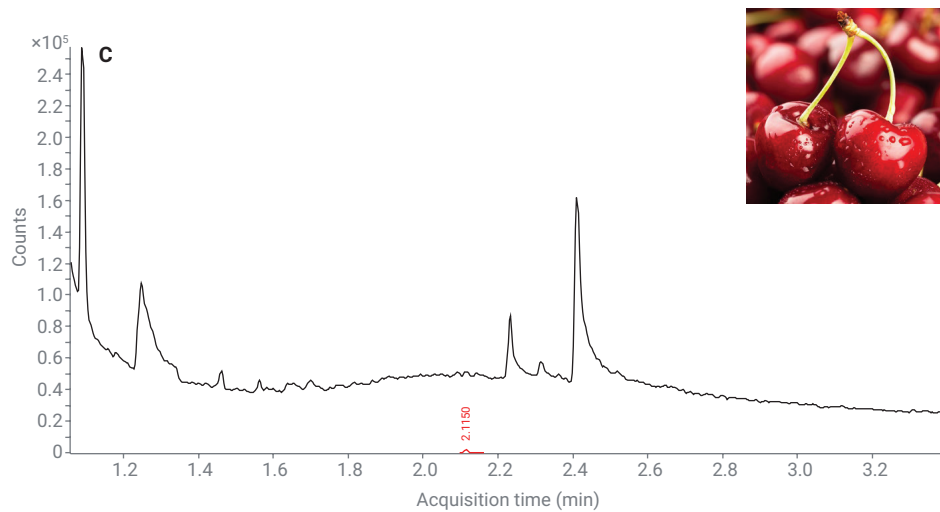


| Component RT | Library RT | Delta RT | Compound Name | Match Factor | Best Hit | Formula | Component Area |
|--------------|------------|----------|--------------------------|--------------|-------------------------------------|-------------|----------------|
| 1.6035 | 1.5805 | -0.0230 | o-Phenylphenol | 61.8 | <input checked="" type="checkbox"/> | C12H10O | 1334.6 |
| 1.6376 | 1.8044 | 0.1667 | Di-n-butylphthalate | 66.0 | <input checked="" type="checkbox"/> | C16H22O4 | 1350.7 |
| 1.7717 | 1.9034 | 0.1316 | Diamyl phthalate | 62.6 | <input checked="" type="checkbox"/> | C18H26O4 | 698.8 |
| 1.8183 | 1.8044 | -0.0139 | Di-n-butylphthalate | 78.8 | <input checked="" type="checkbox"/> | C16H22O4 | 5418.2 |
| 1.9073 | 1.8537 | -0.0536 | Thiabendazole | 96.7 | <input checked="" type="checkbox"/> | C10H7N3S | 405205.6 |
| 1.9614 | 1.9284 | -0.0330 | Buprofezin | 75.0 | <input checked="" type="checkbox"/> | C16H23N... | 12812.3 |
| 2.1076 | 2.0862 | -0.0215 | Bifenthrin | 74.2 | <input checked="" type="checkbox"/> | C23H22Cl... | 3833.6 |
| 2.1461 | 2.1285 | -0.0176 | Bis(2-ethylhexyl)phth... | 63.1 | <input checked="" type="checkbox"/> | C24H38O4 | 4977.6 |
| 2.7538 | 2.6818 | -0.0720 | Azoxystrobin | 89.3 | <input checked="" type="checkbox"/> | C22H17N... | 130424.7 |

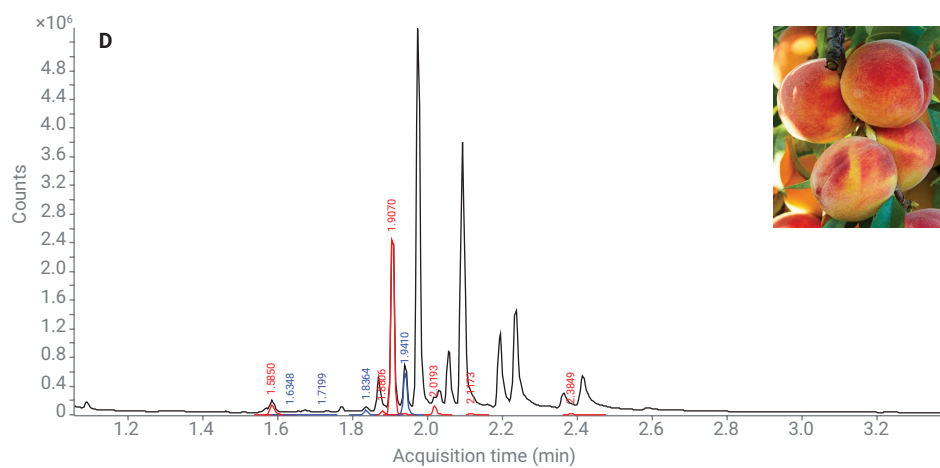


| Component RT | Library RT | Delta RT | Compound Name | Match Factor | Best Hit | Formula | Component Area |
|--------------|------------|----------|-------------------------|--------------|-------------------------------------|-------------|----------------|
| 1.3508 | 1.4072 | 0.0564 | 4-Isopropylaniline | 58.5 | <input checked="" type="checkbox"/> | C9H13N | 304.7 |
| 1.5885 | 1.5756 | -0.0128 | Cashmeran | 61.6 | <input checked="" type="checkbox"/> | C14H22O | 66621.3 |
| 1.6373 | 1.8044 | 0.1670 | Di-n-butylphthalate | 66.3 | <input checked="" type="checkbox"/> | C16H22O4 | 551.8 |
| 1.6663 | 1.6501 | -0.0161 | Chlorpropham | 57.2 | <input checked="" type="checkbox"/> | C10H12Cl... | 2240.5 |
| 1.6883 | 1.6877 | -0.0006 | Empenthrin IV (CAS ... | 56.4 | <input checked="" type="checkbox"/> | C18H26O2 | 186521.1 |
| 1.7366 | 1.7399 | 0.0032 | Exaltolide [15-Penta... | 62.5 | <input checked="" type="checkbox"/> | C15H28O2 | 22674.1 |
| 1.8032 | 1.7902 | -0.0131 | Musk Ambrette | 71.1 | <input checked="" type="checkbox"/> | C16H28O2 | 13633.6 |
| 1.8188 | 1.8044 | -0.0144 | Di-n-butylphthalate | 66.8 | <input checked="" type="checkbox"/> | C16H22O4 | 3270.9 |
| 1.9092 | 1.8537 | -0.0555 | Thiabendazole | 88.9 | <input checked="" type="checkbox"/> | C10H7N3S | 55167.9 |
| 1.9448 | 1.9161 | -0.0286 | Fludioxonil | 96.4 | <input checked="" type="checkbox"/> | C12H6F2... | 782955.8 |
| 1.9502 | 1.9092 | -0.0409 | Imazalil | 65.6 | <input checked="" type="checkbox"/> | C14H14Cl... | 17839.8 |
| 2.1470 | 2.1097 | -0.0373 | Dicyclohexyl phthalate | 59.6 | <input checked="" type="checkbox"/> | C20H26O4 | 4686.6 |
| 2.2971 | 2.4582 | 0.1611 | Di-n-nonyl phthalate | 65.0 | <input checked="" type="checkbox"/> | C26H42O4 | 1401.2 |
| 2.7554 | 2.6818 | -0.0736 | Azoxystrobin | 89.6 | <input checked="" type="checkbox"/> | C22H17N... | 295826.5 |

Figure 7A,B. Screening results for banana (A) and lemon (B) rinsates identified against the pesticide library.



| Component RT | Library RT | Delta RT | Compound Name | Match Factor | Best Hit | Formula | Component Area |
|--------------|------------|----------|---------------|--------------|-------------------------------------|-------------|----------------|
| 2.1150 | 2.0849 | -0.0301 | Fluxapyroxad | 69.0 | <input checked="" type="checkbox"/> | C18H12F5... | 1272.8 |



| Component RT | Library RT | Delta RT | Compound Name | Match Factor | Best Hit | Formula | Component Area |
|--------------|------------|----------|--------------------------|--------------|-------------------------------------|-------------|----------------|
| 1.9070 | 1.8585 | -0.0485 | Captan | 93.2 | <input checked="" type="checkbox"/> | C9H8Cl3N... | 1679701.3 |
| 1.5850 | 1.5607 | -0.0243 | cis-1,2,3,6-Tetrahydr... | 92.1 | <input checked="" type="checkbox"/> | C8H9NO2 | 126991.5 |
| 2.0193 | 2.0155 | -0.0038 | Trifloxystrobin | 90.3 | <input checked="" type="checkbox"/> | C20H19F3... | 95054.7 |
| 1.8806 | 1.8633 | -0.0173 | Fluopyram | 76.7 | <input checked="" type="checkbox"/> | C16H11Cl... | 43718.3 |
| 2.1173 | 2.0849 | -0.0324 | Fluxapyroxad | 76.1 | <input checked="" type="checkbox"/> | C18H12F5... | 13847.8 |
| 1.8364 | 1.7399 | -0.0965 | Exaltolide [15-Penta... | 74.7 | <input checked="" type="checkbox"/> | C15H28O2 | 51549.8 |
| 1.9095 | 1.8795 | -0.0300 | Pyrene | 63.0 | <input checked="" type="checkbox"/> | C16H10 | 1852.8 |
| 1.6348 | 1.6205 | -0.0144 | Diethyl phthalate | 60.6 | <input checked="" type="checkbox"/> | C12H14O4 | 1380.7 |
| 1.5562 | 1.5929 | 0.0367 | Isoprocab | 60.4 | <input checked="" type="checkbox"/> | C11H15NO2 | 1205.7 |
| 1.6091 | 1.6985 | 0.0894 | 4-Aminodiphenyl | 57.5 | <input checked="" type="checkbox"/> | C12H11N | 477.2 |
| 2.3849 | 2.3091 | -0.0758 | Fenbuconazole | 56.4 | <input checked="" type="checkbox"/> | C19H17Cl... | 22035.1 |
| 1.9410 | 1.7902 | -0.1508 | Musk Ambrette | 55.7 | <input checked="" type="checkbox"/> | C16H28O2 | 421548.5 |
| 1.7199 | 1.7557 | 0.0358 | Silthiopham | 55.1 | <input checked="" type="checkbox"/> | C13H21N... | 1428.1 |

Figure 7C,D. Screening results for cherry (C) and peach (D) rinsates identified against the pesticide library.

Identity confirmation with increased chromatographic resolution

To increase confidence in compound identification, the number of coeluting components causing chromatographic and spectral interference can be minimized by using a slower oven ramp. This approach leads to longer chromatographic analysis times, increase in chromatographic resolution, less coelution, and can be used for confirmation purposes. A slower oven ramp rate of 20 °C/min with a total analysis time of 15 minutes was applied to the peach rinsate. The results were searched against the pesticide library using an updated RI calibration, which was created with the slower oven temperature program of 20 °C/min (Figure 8). As expected, LMS for some compounds such as fluopyram and fenbuconazole were improved from 76.7 to 88.5 and from 56.4 to 66.3, respectively, with a slower oven ramp rate due to the decreased interferences.

System robustness

To demonstrate the robustness of the system, 210 injections of a peach rinsate were performed. Additionally, analysis of an *n*-alkane ladder was performed after every 15 injections of a peach rinsate, yielding a total of 235 injections. Chromatograms of a linear *n*-alkane ladder before peach rinsate injections, after 210 injections of peach rinsate, and after liner and septum replacement are shown in Figure 9.

As expected with any GC/MS system, multiple sequential injections of a sample led to response loss, especially pronounced for high-boiling compounds, and an RT shift towards earlier times. Inlet maintenance that included liner and septum replacement recovered most of the response, as shown in Figure 9. An RT shift observed after 210 injections of peach rinsate and an additional 25 injections of an *n*-alkane standard was -0.018 minutes for a

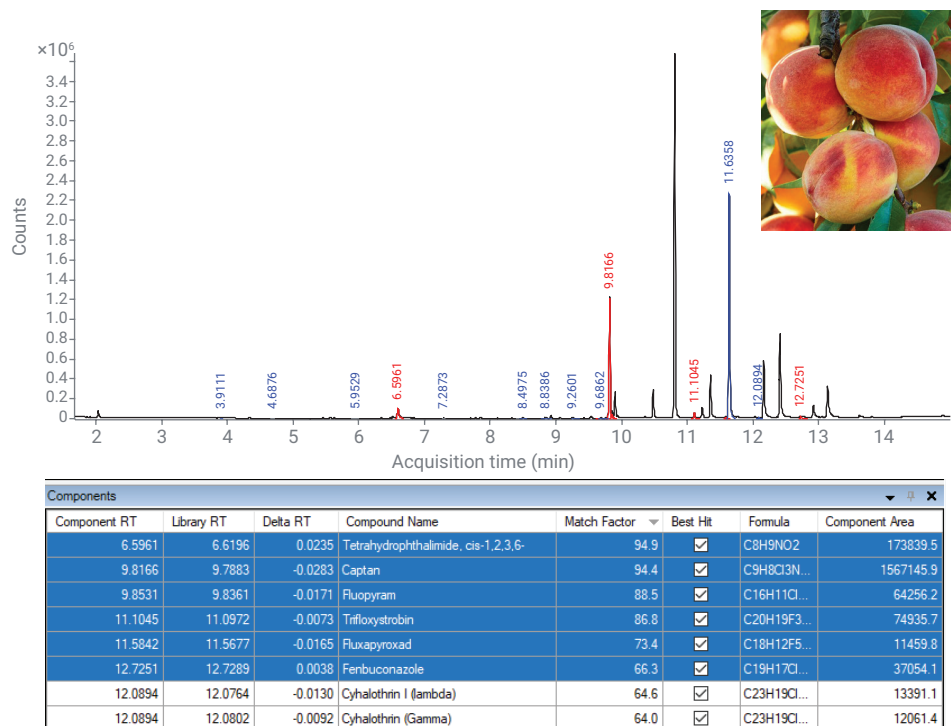


Figure 8. Screening results for peach rinsate identified against the pesticide library with a 15 minute run for improved chromatographic resolution.

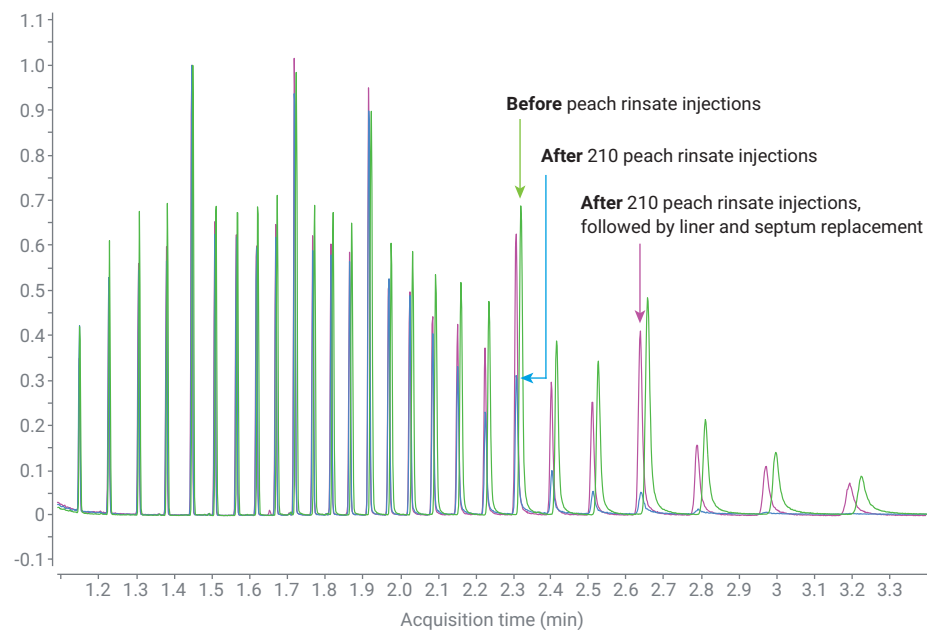


Figure 9. Linear *n*-alkane ladder C₉ to C₃₄ analyzed before peach rinsate injections (green), after 210 injections of peach rinsate (blue), and after liner and septum replacement (purple) normalized by C₁₃.

high-boiling *n*-alkane C₃₁. This RT shift value is comparable or smaller than the delta RT observed in screening result reports; thus, a confident identification with RT filtering would still be possible. Alternatively, the RI calibration can be updated when an *n*-alkane standard is analyzed. This allows for maintaining a relevant RI calibration throughout the lifetime of the system by injecting an alkane standard once every several hundred samples or whenever necessary.

Figure 10 shows the *n*-alkane ladder before and after Guard Chip replacement, which was performed after multiple fruit rinsates had been analyzed. In a conventional GC, column trimming would be a necessary maintenance procedure. However, with the Agilent Intuvo 9000 GC, no column trimming is needed. Instead, the Guard Chip, which serves as a guard column, is replaced. With regular replacement, sample contamination does not reach the head of the Intuvo GC column, thereby eliminating the need for column trimming. Guard Chip replacement is fast, consistent, and simple, minimizing disruption to lab efficiency. Moreover, unlike column trimming, it does not alter the GC flow path and keeps the existing RI calibration relevant with no need for updating. An *n*-alkane ladder analyzed before and after Guard Chip replacement is shown in Figure 10. Note that the RTs of the alkanes remained intact. As expected, Guard Chip replacement recovered the analyte response.

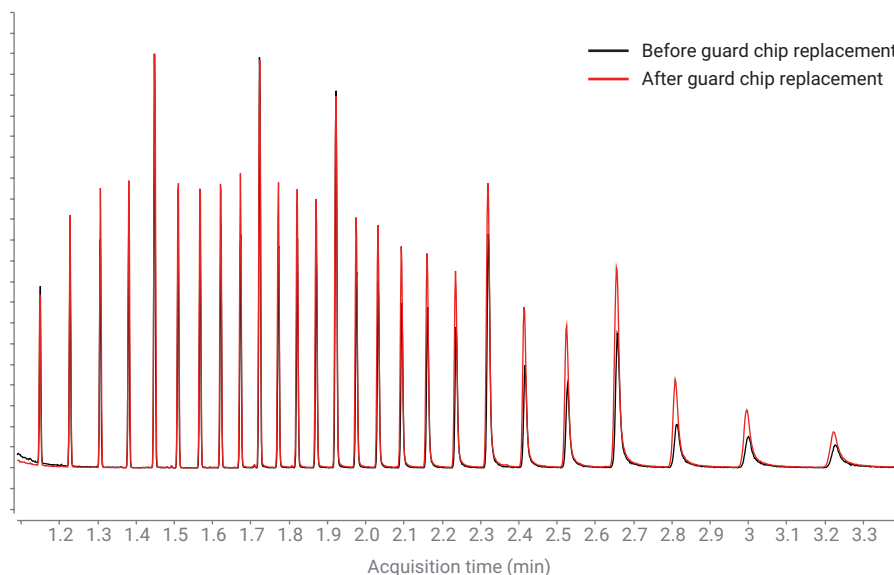


Figure 10. RI Calibration with an *n*-alkane ladder C₉ to C₃₄ before and after Guard Chip replacement normalized by C₁₃.

Conclusion

The Intuvo 9000/5977B GC/MSD system enables rapid screening for pesticides and other contaminants found on the surface of fruits and berries in 3.4 minutes. There is no need to continue the chromatographic run past 3.4 minutes as no pesticides are expected to elute beyond this time. The total workflow time is under 6 minutes, including sample preparation, GC/MSD analysis with backflush, data processing, and reporting. The Intuvo 9000 GC provides oven ramping at a rate of 250 °C/min without requiring special electrical service (V or A) at the bench. This, along with a small footprint of the GC, makes it amenable for use outside of a conventional laboratory setup.

Rapid and reliable identification of pesticides is achieved by library searching of deconvoluted spectra coupled with time-filtering using RIs. The Intuvo 9000 Guard Chip extends column lifetime, and its replacement does not alter RI calibration. Backflushing extends maintenance-free uptime and ensures that no carryover is observed, eliminating the need for extended column bakeout.

The screening workflow described in this application note should be useful for triaging the samples in high-throughput facilities such as receiving docks and distribution centers.

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