Application Note

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# Fast Analysis of Human Malodor Compounds, Volatile Organic Acids by SPME and Column Backflushing


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## Abstract

Solid phase microextraction (SPME) when coupled with gas chromatography/mass spectrometry (GC/MS) offers a solvent-free sample preparation technique that is well suited for the analysis of volatile compounds in difficult matrices. While SPME analysis typically offers less matrix impact than traditional liquid injection, the analysis of human malodor compounds absorbs higher boiling, larger matrix compounds. This analysis requires the use of high temperature bake-out programs that lengthen analysis times and require columns with higher temperature limits. Unfortunately, these higher temperature columns do not provide the selectivity and peak shape needed for optimal quantitation. This application note demonstrates how implementing midcolumn backflushing allows the removal of unwanted column matrix, lessening the need for high bake-out temperatures. This backflushing delivers faster run times and the use of optimal column chemistries, resulting in a more reliable and productive analysis of human malodor compounds by SPME and GC/MS.

## Introduction

Human body odor is caused by the metabolism of sweat by a host of microorganisms in the human underarm. Human sweat is odorless and certain microbial species metabolize secreted sweat components into volatile, malodorous compounds. These bacterial species exist as a microbiome community. Some members of this community can enzymatically convert nonodorous components of human sweat into malodorous compounds, while others are not responsible for creating noxious smells. The human axillary hosts a wide array of bacterial species, including *Corynebacterium jeikeium* and *Corynebacterium tuberculostearicum* along with *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus hominis*.[1] The relative population of each species within the microbiome community influences the number of malodorous compounds liberated from digested human sweat. Typically, a variety of volatile organic acids (VOAs) are produced and are comprised of long-chain fatty acids, fatty acids bound to amino acids, and sulfur-containing amino acids. However, several studies suggest that only a few, key odorants are predominately responsible for the negative human perception of body odor, which include E-3-methyl-2-hexenoic acid (E-3M2H), isovaleric acid, 3-methyl-3-sulfanylhexan-1-ol (3M3SH), and 3-hydroxy-3-methylhexanoic acid (HMHA).[2]

Traditional deodorant products are formulated to reduce or prevent the occurrence of axillary malodor. Deodorant products also can include fragrances to mask noxious compounds, with or without added antimicrobials. Antiperspirants, which may also include antimicrobial ingredients and fragrances, work to reduce or prevent sweat. These products can reduce the otherwise favorable conditions for bacterial growth in the axillary region.

Research indicates that the use of antiperspirants containing aluminum salts can affect axillary microbiome diversity in the axillary region of a person. These conventional products, while generally effective in preventing malodors, might also result in major changes to the axillary microbiota of the user. ScentARC[3], launched by Arcaea, is meant to biologically shift our human scent in a natural and precise manner. ScentARC is incorporated into a scentless deodorant that changes the user's odor profile by selectively and naturally shifting the underarm microbiome community. This shift is in favor of multiple bacterial species that do not metabolize sweat into malodorous compounds.

While optimizing ScentARC formulation blends, an in vitro model was developed to mimic the axillary region of the human underarm using distinct species of bacteria isolated from human underarms. This model was used to screen different base formulations for deodorant efficacy through measuring the amount of malodorous volatile compounds produced by the bacterial community after incubation with the ScentARC blend, relative to a control community. Due to the complexity of biological sample matrices, headspace SPME GC/MS was chosen for analysis to minimize instrument contamination, as well as time-consuming sample preparation.

SPME is a technique invented in the early 1990s, where a fiber coated in an absorbent polymer phase is exposed to a sample, either by direct immersion or in the headspace above the sample (Figures 1 and 2).[4] The target analytes are adsorbed from the sample to the phase, depending on affinity to the phase, and once reaching equilibrium, will be removed from the sample and desorbed onto the inlet of the GC. These methods of sample preparation and sample introduction have been especially useful in the analysis of low concentration compounds in volatile compounds and in decreasing the effects of heavy matrices.

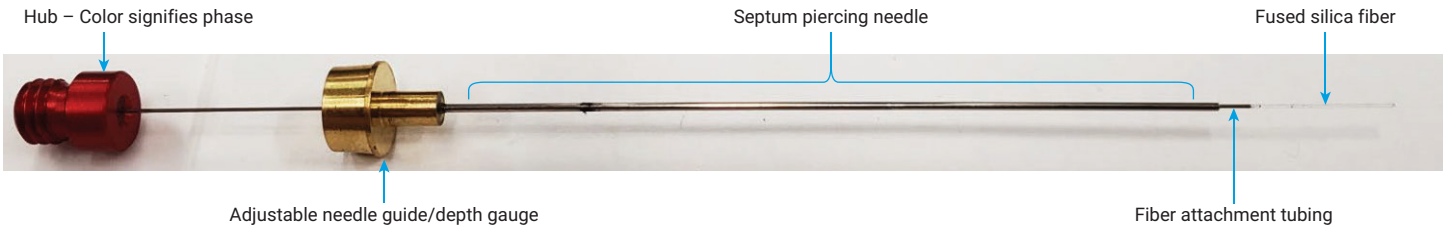


**Figure 1.** Diagram of an SPME fiber.

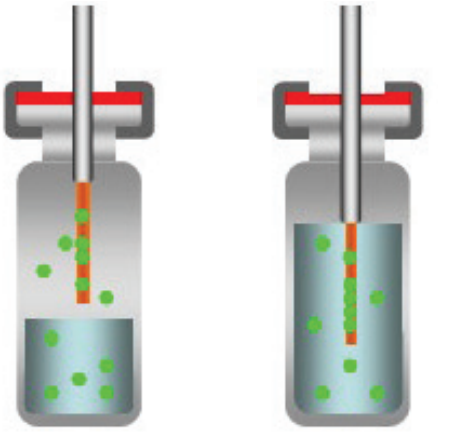


**Figure 2.** Example of headspace sampling versus direct immersion using SPME sampling technique.

Another technique that can be used when working with heavy matrix samples is the use of backflushing. Capillary column backflush is accomplished by a reversal of flow through the column so that some of the elements are forced back out the inlet, typically the split vent. Midcolumn backflushing was used in this method to eliminate the need for a column bake-out, which drastically reduced the run time. Midcolumn backflushing allows the removal, from the column, of unwanted high boilers in a sample. This removal works without the drawbacks of exposing a column to high temperatures and long runtimes encountered with a traditional bake-out approach. Midcolumn backflushing eliminates the need for higher temperatures or longer hold times for unwanted higher boiling compounds or other heavy matrix components to elute off of the column. The use of midcolumn backflush not only decreases the overall GC oven runtime but extends the lifetime of the column; it also extends the time between source cleanings, since higher temperatures to elute higher boiling compounds are no longer required.[5,6]

Without the need for higher temperatures, column phase selection can be based on which column phase provides the optimum peak shape, instead of which phase provides the maximum allowable operating temperature (MAOT) required to elute the highest boiling compounds or heavy matrix in a sample. The MAOT of a column phase is specified as the highest temperature to which a GC column can be exposed without damaging the thermal stability of the column. Nonpolar columns, such as Agilent J&W DB-5ms UI GC columns will have a higher MAOT than polar GC columns, such as Agilent J&W DB-Wax UI GC columns. The higher MAOT is an indicator of thermal stability, not whether a phase is suitable for analysis of a specific compound. Compounds such as organic acids will display poor peak shape on nonpolar column phases, but when analyzed on a polar column phase will display optimum peak shape. This is why it is important to select the optimum GC column phases to analyze specific compounds of interest.[7,8]

Determining which GC column phase to select can be difficult when analyzing trace level aromatic compounds. Spectral deconvolution can be helpful in removing ions of coeluting compounds and in the identification of all peaks. The Agilent MassHunter Quantitative Analysis software with the Unknowns Analysis program is a tool that can be used to analyze multiple samples, deconvolute spectra, and compare retention times or retention index (RI) commercially available libraries such as NIST or user-created libraries.[9]

To demonstrate the benefit of combining these techniques, the application of analyzing malodor compounds was optimized to improve overall data quality and productivity.

# Materials and methods

## Target volatiles

Analytical standards of 3-methyl-2-hexenoic acid (E-3M2H and Z-3M2H isomers) and 3-methyl-3-sulfanylhexan-1-ol (3M3SH) were purchased from Sigma and prepared to a concentration of 1 mg/mL in LC/MS grade methanol. The standards were further diluted to concentrations of 0.2, 2.0, and 20 ppm using a sweat-like medium. Vials were prepared with a 2 mL sample in a 20 mL headspace vial, capped, incubated for 18 hours, then immediately analyzed by GC/MS.

## Method

A Gerstel MPS Robotic Pro was installed on an Agilent 8890 GC system with an Agilent 5975 MSD. The SPME headspace parameters and MS conditions are listed in Tables 1 through 4. Data were analyzed using Agilent MassHunter Unknowns Analysis.

**Table 1.** Gerstel MPS Robotic Pro parameters.

| Parameter | Setting |
|---|---|
| Fiber | Agilent SPME fiber, DVB/C-WR/PDMS (p/n 5191-5874) |
| Predesorption Time | 5.0 min |
| Predesorption Temperature | 70 °C |
| Incubation Time | 5.0 min |
| Agitation Speed | 500 rpm |
| Sample Extraction Time | 15.0 min |
| Sample Desorption Time | 180 s |

**Table 2.** Agilent 8890 GC method A settings.

| Parameter | Value |
|---|---|
| **Agilent 8890** | |
| Inlet | 250 °C, Splitless mode, multimode inlet (MMI) |
| Inlet Liner | Agilent Inlet liner, Ultra Inert, splitless, straight, 2 mm id (p/n 5190-6168) |
| Purge Flow to Split Vent | 20 mL/min at 2.0 min |
| Septum Purge Flow | 3 mL/min |
| Oven | 40 °C (5 min),<br>Ramp 10 °C/min to 136 °C,<br>Ramp 20 °C/min to 300 °C (4.0 min) |
| **Column** | |
| Carrier Gas | Helium |
| Column | Agilent DB-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532UI) |
| Inlet Connection | Multimode Inlet (MMI) |
| Outlet Connection | MSD |

**Table 3.** Agilent 8890 GC method B settings.

| Parameter | Value |
|---|---|
| **Agilent 8890** | |
| Inlet | 250 °C, Solvent vent mode, multimode inlet (MMI) |
| Inlet Liner | Agilent Inlet liner, Ultra Inert, splitless, straight, 2 mm id (p/n 5190-6168) |
| Purge Flow to Split Vent | 15 mL/min at 0.35 min |
| Septum Purge Flow | 3 mL/min |
| Vent Flow | 100 mL/min |
| Vent Pressure | 5 psi until 0.25 min |
| Oven | 60 °C (1 min),<br>Ramp 40 °C/min to 170°C,<br>Ramp 10 °C/min to 250 °C (0.5 min) |
| Carrier Gas | Helium |
| **Column 1** | |
| Column | Agilent DB-Wax UI, 15 m × 0.25 mm, 0.25 µm (p/n 122-7012UI) |
| Flow | 1 mL/min, constant flow |
| Inlet Connection | Multimode inlet (MMI) |
| Outlet Connection | PSD (purged 2-way splitter) (G3180-60501) |
| PSD Purge Flow | 1 mL/min |
| Postrun Flow (Backflushing) | –6.943 mL/min |
| **Column 2** | |
| Column | Agilent DB-Wax UI, 15 m × 0.25 mm, 0.25 µm (p/n 122-7012UI) |
| Flow | 1.2 mL/min, constant flow |
| Inlet Connection | PSD (purged 2-way splitter) (G3180-60501) |
| Outlet Connection | MSD |
| Postrun Flow | 7.3775 mL/min |

**Table 4.** Agilent 5977B MSD conditions.

| Parameter | Value |
|---|---|
| Source | XTR |
| Mode | Scan |
| Solvent Delay | 3.0 min |
| Source Temperature | 230 °C |
| Quad Temperature | 150 °C |
| Gain | 1.0 |

**Table 5.** Flow path supplies.

| Parameter | Value |
|---|---|
| Septum | Bleed and temperature optimized (BTO), 11 mm septa (p/n 5183-4757, 50/pk) |
| Inlet/MSD | V/G ferrules (p/n 5181-3323) |
| Column Nut | – Collared, self-tightening column, inlet (p/n G3440-81011)<br>– Collared, self-tightening column, MSD (p/n G3440-81013)<br>– Internal nut, CFT capillary fitting (p/n G2855-20530) |
| PSD | – CFT ferrule flex gold 0.25 mm id (p/n G2855-28501)<br>– Plug for microfluidic union (p/n G2855-60570)<br>– Column pre-swaging tool (p/n 2855-60200) |

## Results and discussion

### Selecting the optimal column phase

Figure 3 shows the scan total ion chromatogram (TIC) of a three-component reference mixture initially analyzed on a J&W DB-5ms UI GC column using GC method A. Using MassHunter Unknowns Analysis, the TIC was deconvoluted and peak identification was made with the NIST20 Library. It was found that two analytes of interest, isomers of 3M2H (Z-3M2H and E-3M2H), coeluted and had poor peak shape on the DB-5ms UI, as demonstrated in Figure 4. The DB-5ms UI was initially chosen due to the requirement of a higher final temperature to elute all compounds off of the column. The final temperature of 300 °C with a hold time of 4.0 minutes was an acceptable temperature for the DB-5ms UI GC column. However, the coelution and peak shape proved to be problematic for quantification and identification. Due to all analytes of interest eluting before reaching a temperature of 250 °C, it was possible to use a more polar column phase to provide better peak shape.

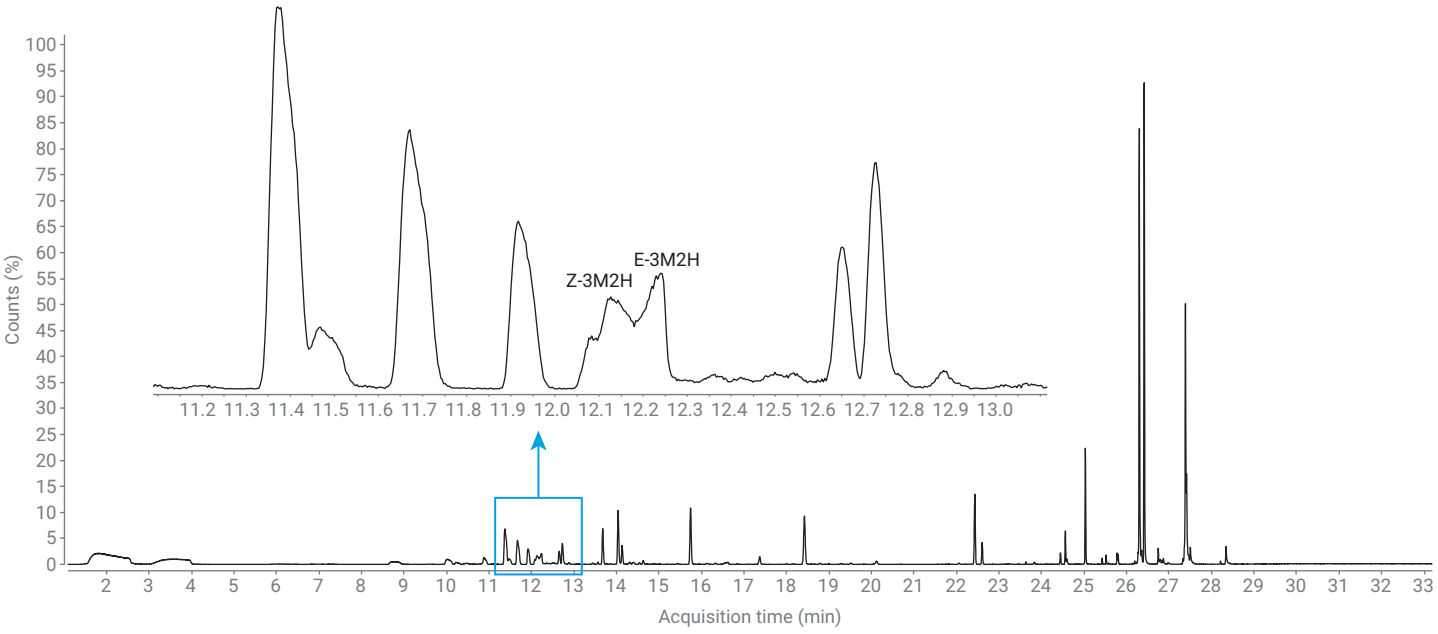


**Figure 3.** Standard of a three-component reference mixture spiked into a matrix blank and analyzed on an Agilent DB-5ms UI GC column using GC method A.

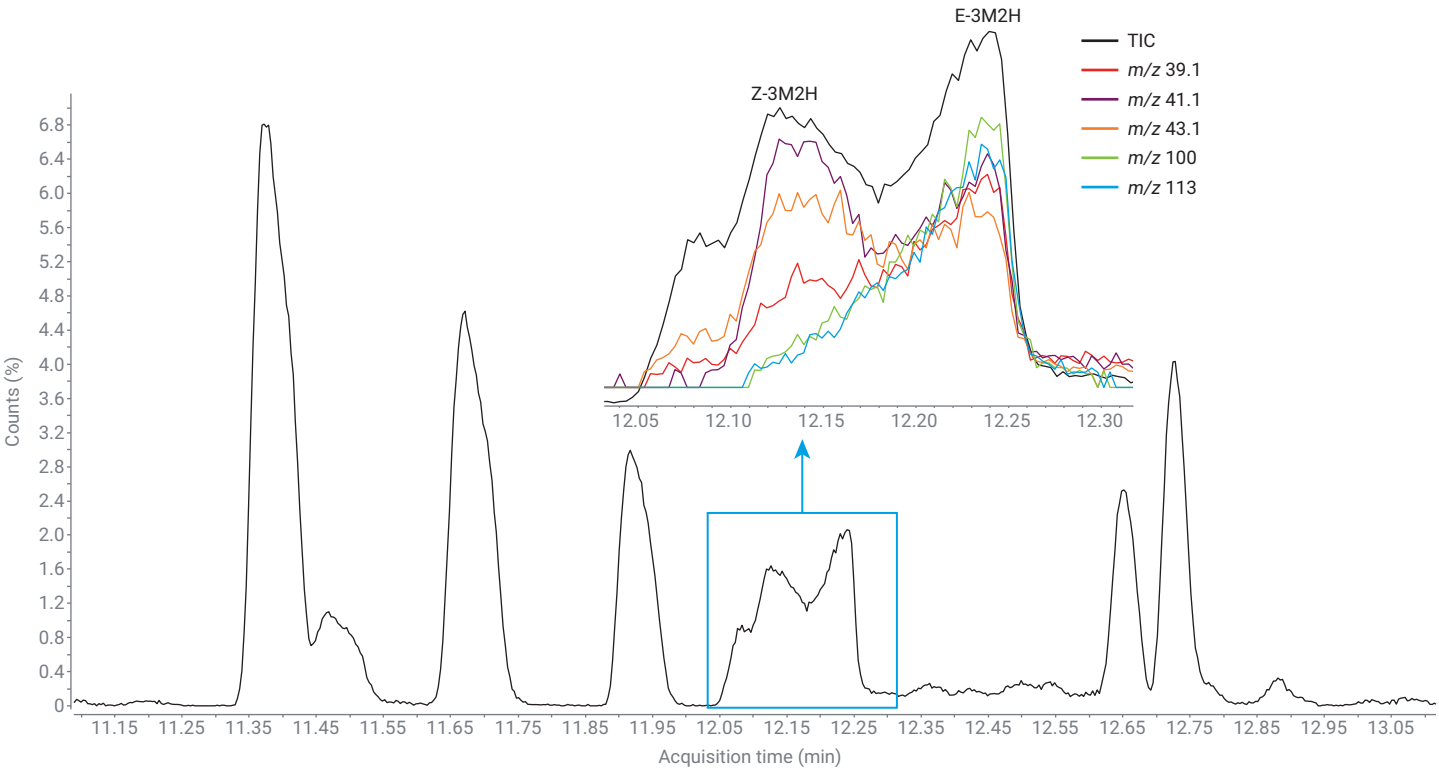


**Figure 4.** Standard of a three-component reference mixture analyzed on an Agilent DB-5ms UI GC column using GC method A. The zoom shows extracted ions for identification.

## Implementing and using backflushing

A J&W DB-Wax UI GC column was selected due to the polarity of the column phase, and to provide better peak shape for polar, volatile organic compounds. Two 15 m × 0.25 mm, 0.25 µm DB-Wax UI columns were installed, with column 1 connected from the inlet to the purged 2-way splitter plate. Column 2 was connected from the purged 2-way splitter plate to the MSD transfer line, and a CFT plug was installed into the unused connection point on the splitter plate, as shown in Figure 5. An initial run using GC method B was set up using a 15-minute hold time after the oven had reached 250 °C to ensure that all analytes eluted from the column while not exceeding the MAOT of the DB-Wax UI GC column (Figure 6). It was determined that the analytical run could be stopped after 12 minutes and backflushed for 1.8 minutes. As demonstrated in Figure 7, the subsequent blank after the backflushed sample was clean, and indicated that no matrix remained on the column.

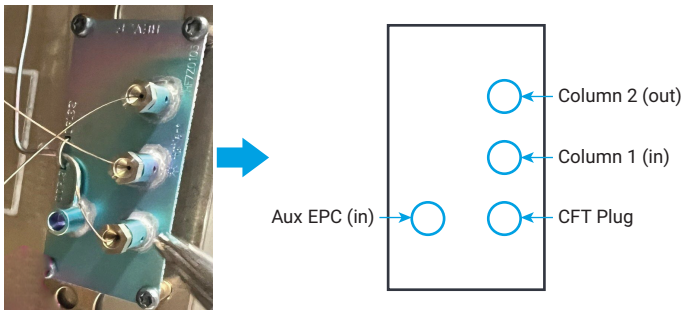


**Figure 5.** Diagram of column installation into and out of the purged 2-way splitter.

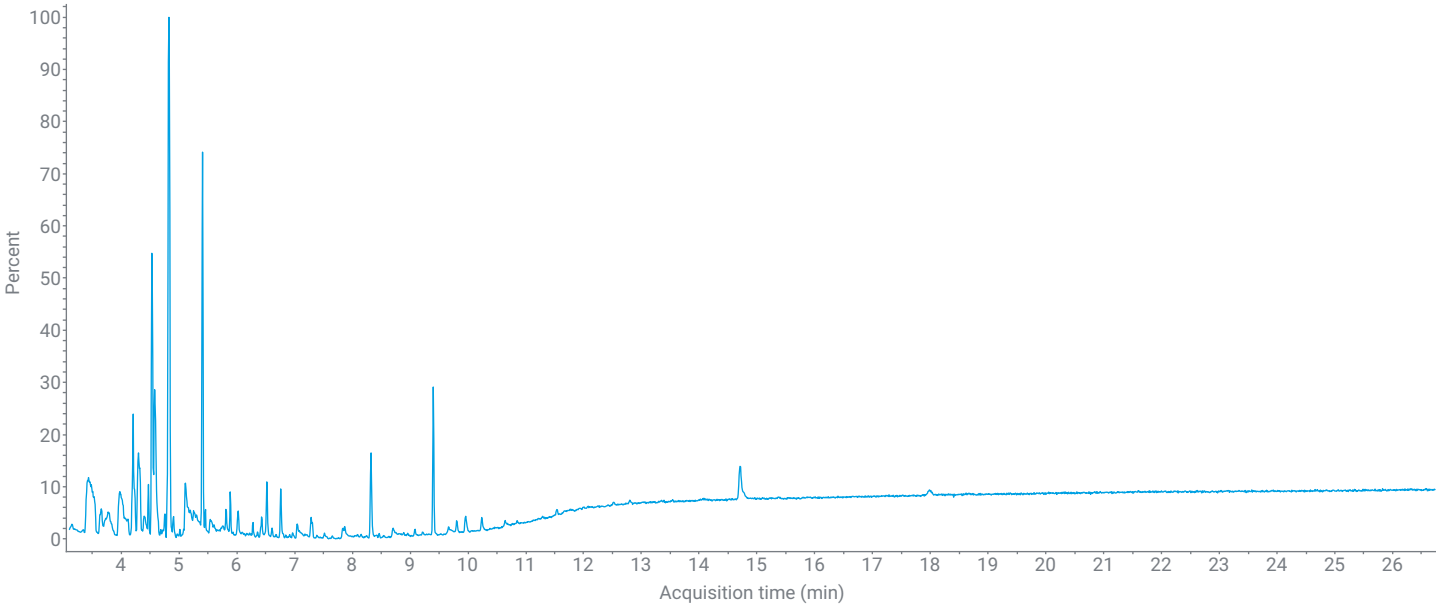


**Figure 6.** Standard of a three-component reference mixture analyzed on an Agilent J&W DB-Wax UI GC column using method B with an extended hold time at 250 °C instead of using a backflush.

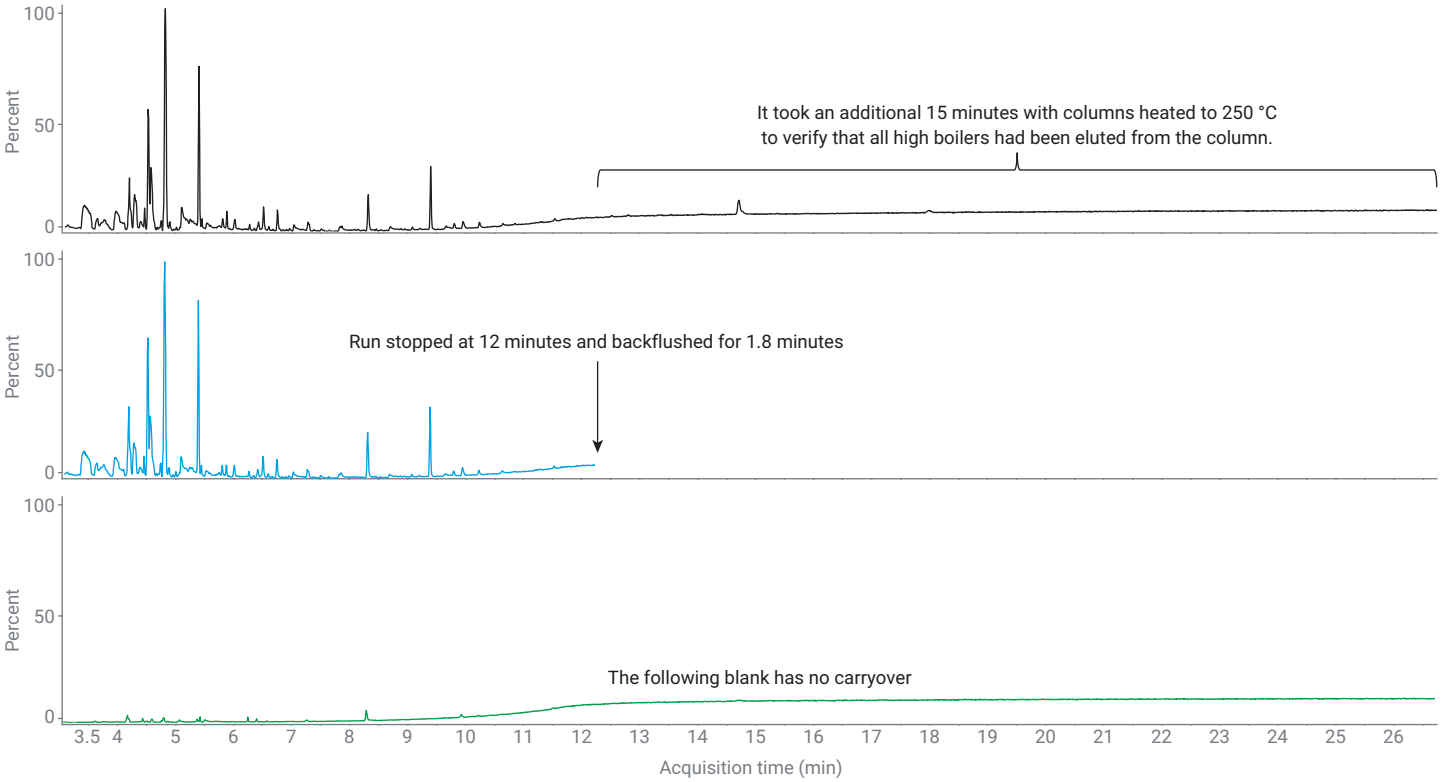


**Figure 7.** The three-component mixture analyzed on an Agilent DB-Wax UI using GC method B demonstrated that the subsequent blank after a backflushed sample did not contain any carryover or heavy matrix.

## Faster deconvolution using MassHunter Unknowns Analysis

To determine if the DB-Wax UI was the optimal column phase for the analysis of these volatile organic compounds, MassHunter Unknowns Analysis was used to deconvolute and search mass spectra against a library database. Once the analytical method was optimized to include midcolumn backflush, the same three-component mix was analyzed on a J&W DB-Wax UI GC column, and deconvoluted using MassHunter Unknowns Analysis. The NIST20 library was selected to search against the deconvoluted spectra, and the compound E-3M2H was easily identified and found to have good peak shape, as demonstrated in Figure 8. When comparing the peak shape of the E and Z isomers collected on the DB-5ms UI (Figure 9A) to the analysis on the DB-Wax UI (Figure 9B) the results demonstrate that there was no longer coelution of the isomers, the peak shape was dramatically improved, and making identification and quantification was easier.

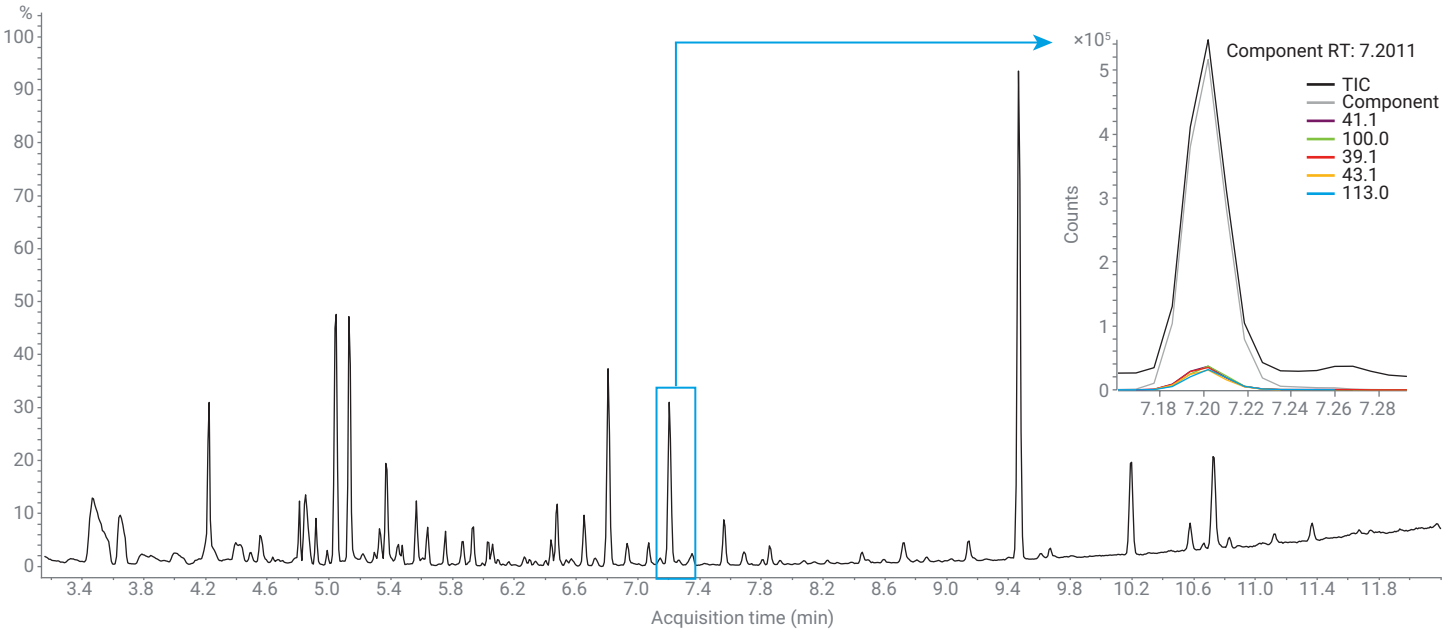


**Figure 8.** The three-component mix analyzed on an Agilent DB-Wax UI column with optimized method parameters including backflush, with E-3-methyl-2-hexenoic acid identified using Agilent MassHunter Unknowns Analysis.

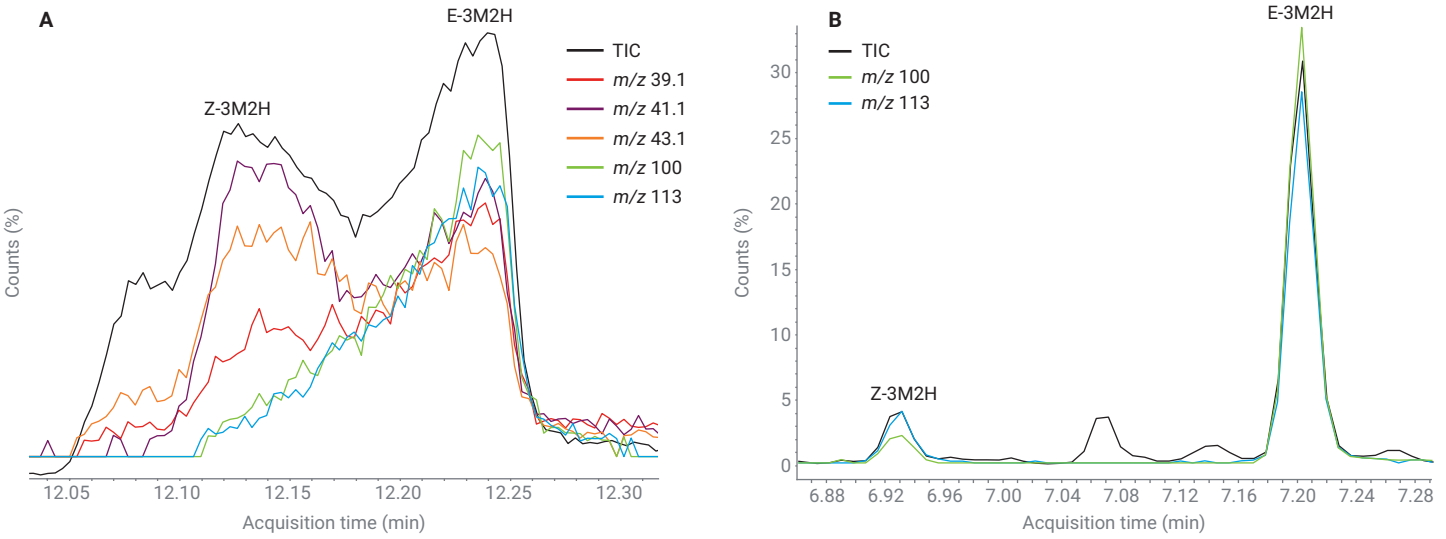


**Figure 9.** Extracted ions of E-3-methyl-2-hexenoic acid and Z-3-methyl-2-hexenoic acid from a three-component mix analyzed on an Agilent DB-5ms UI (A) and an Agilent DB-Wax UI (B) and identified using Agilent MassHunter Unknowns Analysis.

Figure 10 shows the report created after deconvoluting and searching against a library, with two analytes of interest correctly identified in the three-component mix. While this sample was searched against a NIST library, it was also possible to create a user library and search as well. Figure 11 shows the information displayed when inspecting a hit for E-3-methyl-2-hexenoic acid. Figure 11A shows the TIC along with peaks for all potential hits in green. The peaks for E-3M2H are in blue to indicate which peak, along with EICs and deconvoluted spectra, are being compared in Figure 11B and Figure 11C, respectively.

Components

| Component RT | Compound Name | Match Factor | Best Hit | Formula | Targ |
|---|---|---|---|---|---|
| 6.5675 | 1-Phenyl-1-decanol | 81.1 | ☑ | C16H26O | |
| 6.5851 | 2-Propenoic acid, oxiranylmethyl ester | 61.6 | ☑ | C6H8O3 | |
| 6.6511 | 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate | 88.8 | ☑ | C16H30O4 | |
| 6.7186 | 2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohe... | 76.4 | ☑ | C19H32O | |
| 6.7269 | Succinic acid, 3-methylbut-2-yl 2,2,3,3,3-pe... | 73.0 | ☑ | C12H17F5... | |
| 6.7335 | 1-Cyclohexyldimethylsilyloxybutane | 56.3 | ☑ | C12H26OSi | |
| 6.7946 | (R)-(-)-4-Methylhexanoic acid | 68.8 | ☑ | C7H14O2 | |
| 6.8075 | Phenylethyl Alcohol | 98.2 | ☑ | C8H10O | |
| 6.9276 | Z-3-Methyl-2-hexenoic acid | 79.6 | ☑ | C7H12O2 | |
| 7.0057 | 2-Propenoic acid, 2-methyl-, octyl ester | 67.9 | ☑ | C12H22O2 | |
| 7.0636 | Silane, tetramethyl- | 61.9 | ☑ | C4H12Si | |
| 7.0660 | Benzene, 1,2,3,5-tetramethyl-4,6-dinitro- | 56.3 | ☑ | C10H12N... | |
| 7.0684 | 9-[4-[1,3-Diphenyl-2-imidazolidinyl]-2,3-O-[1-... | 55.8 | ☑ | C28H30N... | |
| 7.0701 | 4-Amino-2-methoxy-5-nitrobenzoic acid, N-tri... | 66.4 | ☑ | C14H24N... | |
| 7.0895 | 2-Hydroxy-3-pentanone | 59.2 | ☑ | C5H10O2 | |
| 7.1344 | Anthracen-9-one, 10-heptyl-10-hydroxy- | 67.8 | ☑ | C21H24O2 | |
| 7.1430 | 1-Butanol, 2-methyl- | 87.9 | ☑ | C5H12O | |
| 7.2011 | E-3-Methyl-2-hexenoic acid | 93.7 | ☑ | C7H12O2 | |
| 7.2669 | 1H-Pyrazolo[3,4-d]pyrimidin-4-amine | 65.9 | ☑ | C5H5N5 | |
| 7.3427 | 2-Vinylfuran | 73.0 | ☑ | C6H6O | |
| 7.3511 | Butanoic acid, methyl ester | 59.1 | ☑ | C5H10O2 | |
| 7.3528 | 2-(Oxolan-2-yl)-1,2-oxazinane | 73.8 | ☑ | C8H15NO2 | |
| 7.4315 | 2-Heptanone | 74.5 | ☑ | C7H14O | |
| 7.5578 | 5-Dodecen-1-ol, acetate, (Z)- | 91.3 | ☑ | C14H26O2 | |
| 7.5611 | Urea, 3-(2-chloro-4-nitrophenyl)-1-ethyl-1-(3-f | 56.4 | ☑ | C15H13Cl | |

**Figure 10.** A partial list of the search results for the three-component mix against the NIST20 library.

In this case, the compound E-3-methyl-2-hexenoic acid was correctly identified, producing a high-quality library match score (LMS) of 93.7. Had the LMS not been as high, it would be necessary to examine all possible alternate hits for this deconvoluted spectrum. One benefit to using MassHunter Unknowns Analysis is that each peak and potential hit will have multiple alternate hits, which can easily be examined by the user, and automatically update the search results page. Then, the correct identification will be in the final report table. Additionally, peaks that are deemed incorrect can be deleted from the results table so that only confirmed hits remain in the final report.

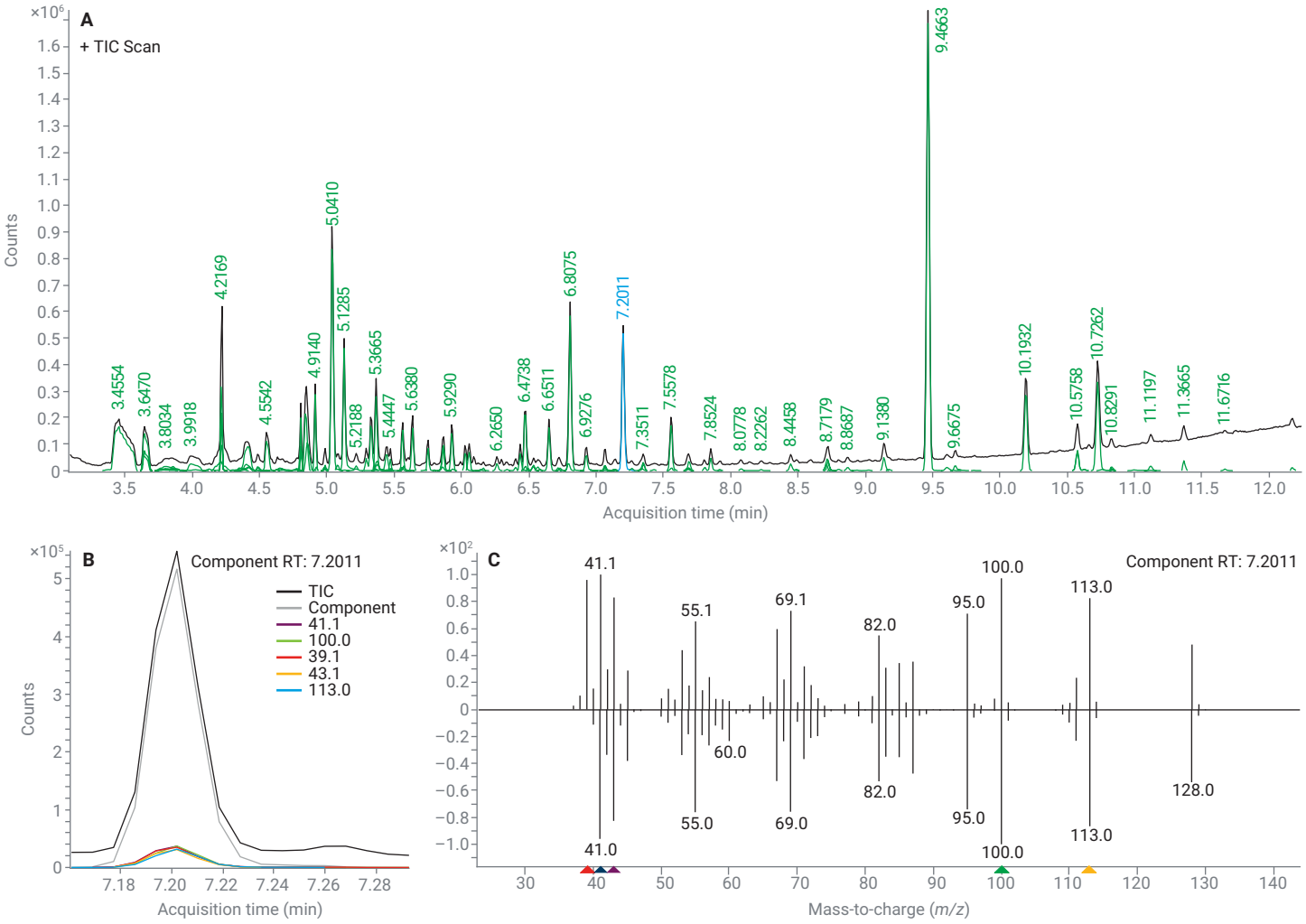


**Figure 11.** Identification of E-3-methyl-2-hexenoic acid (E-3M2H) with Agilent MassHunter Unknowns Analysis.

## Conclusion

The use of solid phase microextraction (SPME) is a valuable sampling technique for measuring volatile organic compounds produced by human skin commensals, especially when considering the complex sample matrix of multispecies in vitro bacterial communities. The use of a polar column phase such as an Agilent J&W DB-Wax UI GC column will provide better peak shape for organic acids than a nonpolar column. When switching to a more polar phase, the implementation of midcolumn backflush will help to remove unwanted high-boiling matrix contamination in the VOA sample at lower oven temperatures. This method allows for faster run times, extended column lifetimes, and reduced contamination of the MS source. The Agilent MassHunter Quantitative Analysis software with Unknowns Analysis can be used to easily deconvolute samples by searching against a commercial library or a user-created library to aid in faster sample analysis. Finally, combining SPME with midcolumn backflushing provides a major improvement in method runtimes compared to other methodologies.

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