Application Note

Flavor and Fragrance

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# Analysis of Coffee Aroma Components with Agilent PAL3 Autosampler and 7010B GC/TQ

## Complementary Agilent sampling techniques of static headspace, dynamic headspace ITEX, SPME, and SPME Arrow


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## Abstract

The Agilent 7010B triple quadrupole GC/MS system (GC/TQ), operating in full-scan data acquisition mode, was used for the qualitative analysis of volatile organic aroma compounds in coffee samples. In this application note, fully automated solvent-free extraction and complementary Agilent sample introduction techniques including static headspace, dynamic headspace ITEX, solid phase microextraction (SPME), and SPME Arrow were evaluated to uncover the complete volatile profile of coffee. Compound identification was performed using deconvoluted mass spectra matched against NIST and Wiley libraries by Agilent MassHunter Unknowns Analysis software. This application note provides the guidelines for data acquisition and processing with the Agilent 7010B GC/TQ, PAL3 autosampler system, and MassHunter Unknowns Analysis software. In this application note, 146 compounds of flavor and fragrance relevance were detected with the four sample introduction techniques. Among these 146 compounds, 6, 48, 27, and 11 compounds were uniquely detected using headspace, dynamic headspace ITEX, SPME, and SPME Arrow, respectively. The rest of the compounds were detected with at least two of the sample introduction techniques.

## Introduction

Coffee is one of the most popular beverages in the world, and it is an important agricultural product in countries such as Brazil, Vietnam, Indonesia, and Colombia.[1] Different varieties of coffee have different chemical profiles, which play an important role in its taste and aroma. As a result, there is great research interest in the aroma composition of coffee. However, the composition of volatiles from coffee is complex, with hundreds of chemical compounds contributing to the aroma. These aroma compounds, including acids, alcohols, aldehydes, esters, furans, pyrazines, pyridine, and thiols[2-4], are correlated to coffee taste and its qualities.

To analyze volatile compounds from coffee, several sample preparation techniques are used: liquid–liquid extraction (LLE)[5], SPME[6], and dynamic headspace extraction (DHS)[7]. These techniques are usually coupled with GC/MS instruments. Among these techniques, LLE is tedious and time-consuming. It requires manual sample preparation with toxic organic solvents. Static headspace and SPME are often operated in manual mode, which is neither productive nor reproducible. DHS, when used as the only sampling technique, is not comprehensive. It does not provide the full picture of compounds in coffee .

Static headspace is a technique that analyzes volatile organic compounds in complex samples that cannot be directly injected into a GC or GC/MS system. Static headspace analysis samples the gaseous form of the compounds that are evaporated from liquid or solid samples in the headspace of the sample vial. If the analyte of interest is not volatile enough at room temperature, heat and agitation can be applied to help reach an equilibrium between the phases. Static headspace offers the advantages of simplified sample preparation, smaller solvent peaks, and reduced instrument maintenance. These improvements are due to a cleaner gas sample with minimal sample matrix being introduced to the GC or GC/MS system.

Dynamic headspace ITEX is a syringe-based technique for rapid and efficient enrichment of volatile and semivolatile compounds from gaseous, liquid, and solid samples. The syringe is filled with Tenax adsorbent material to concentrate and enrich the volatile compounds. The in-tube extraction procedure is shown in Figure 1. The collection of target compounds is through repeated strokes of the syringe. With a higher number of strokes, higher concentration and enrichment can be achieved to improve the detection of trace-level compounds. After sample adsorption, the ITEX tool moves to the GC injector for desorption and analysis.

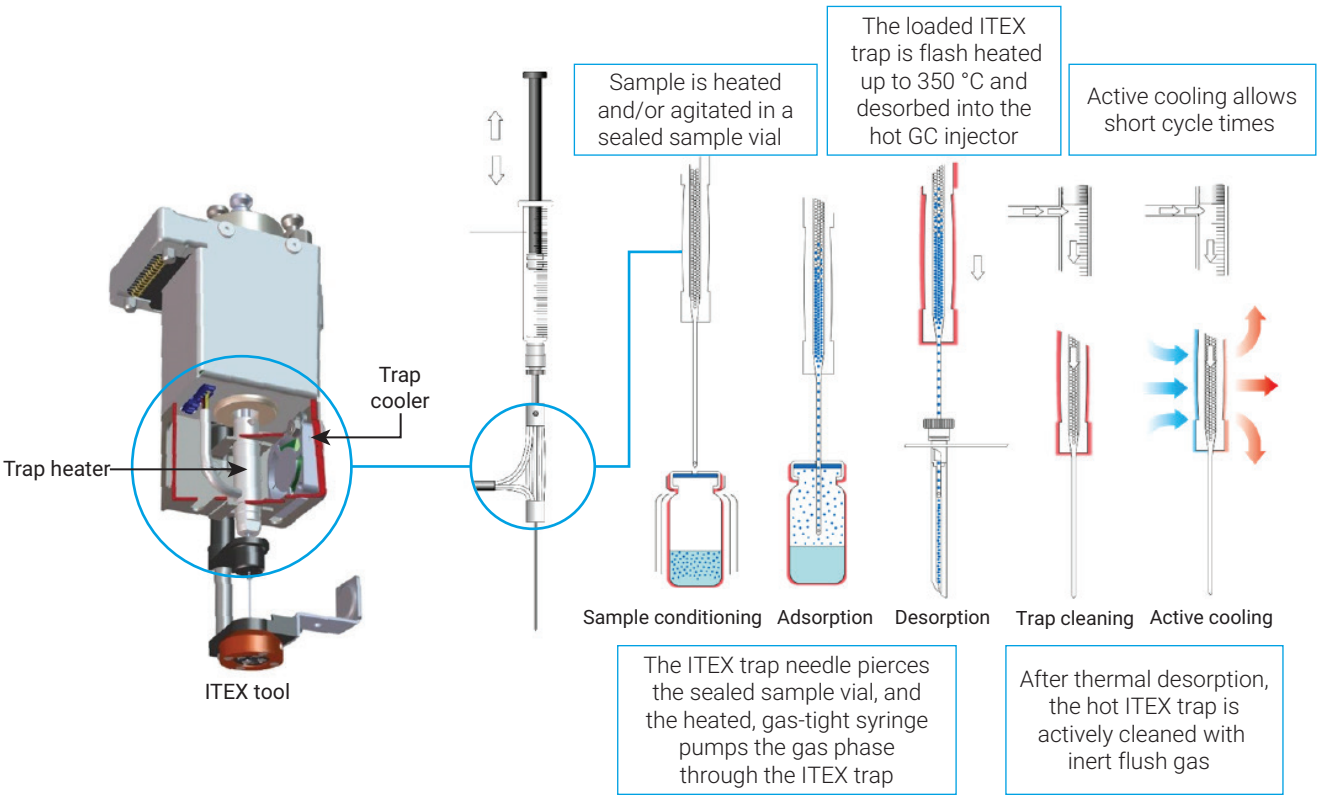


**Figure 1.** Schematic of dynamic headspace ITEX .

SPME is a common solvent-free sample preparation technique. It works on the principle of adsorption and desorption. It uses a fiber coated with an extractive phase to concentrate analytes in a sample. SPME is widely used in many kinds of analyses including environmental, food and flavor, pharmaceutical, etc.

SPME Arrow is a new technology that is based on conventional SPME with the benefits of providing larger sorption-phase surfaces and volumes. There are different kinds of fiber available including PDMS, acrylate, carbon WR, DVB, and different combinations of these four sorbents, to cater to different of analyte polarities. The arrow-shaped tip not only fully protects the sportive material but allows smooth penetration of vial and injector septa. Compared with conventional SPME, SPME Arrow offers two times higher sample throughput and up to 10 times more sensitivity due to its thicker fiber. The schematic of SPME/SPME Arrow is shown in Figure 2. The differences between standard, conventional SPME fibers and SPME Arrow fibers are shown in Figure 3.

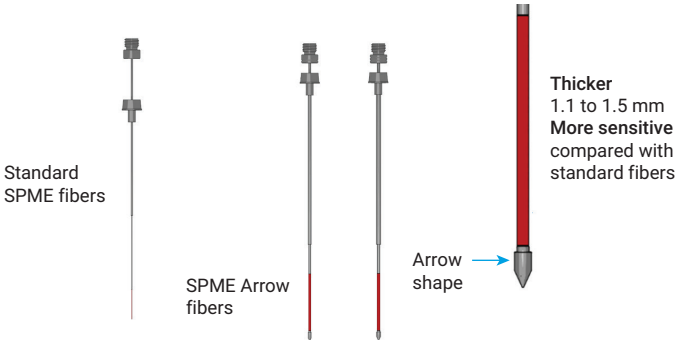


**Figure 3.** Differences between standard SPME fibers and SPME Arrow fibers.

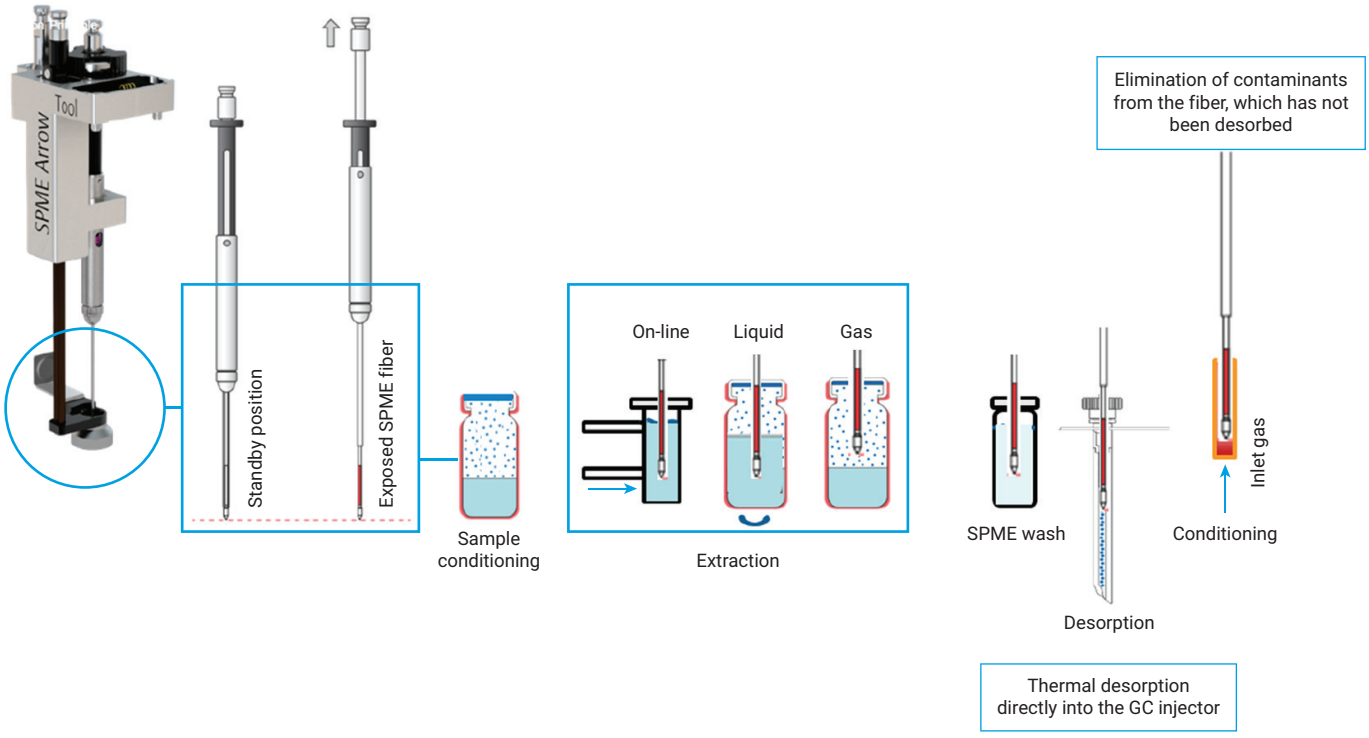


**Figure 2.** Schematic of SPME/SPME Arrow.

GC/TQ is typically operated in multiple reaction monitoring (MRM) mode to achieve high sensitivity through high selectivity for trace-level quantitation analysis. GC/TQ can also be operated in scan mode for full-scan acquisition analysis. With the scan data, library matching can be performed for identification of unknown compounds.

This application note demonstrates the use of the PAL3 RTC autosampler and the 7010B GC/TQ in scan mode for the analysis of the volatile aroma compounds in roasted coffee powder. Four sampling tools including the static headspace tool, dynamic headspace ITEX tool, SPME tool, and SPME Arrow tool (as shown in Figure 4) were used. The PAL3 autosampler offers the advantage of seamless integration and automatic tool change during sample analysis. The four fully automated sample preparation and introduction techniques are complementary in nature and offer full aroma profiling of coffee.


**Figure 4.** (A) Agilent static headspace tool, (B) Agilent dynamic headspace ITEX tool, (C) Agilent SPME tool, (D) Agilent SPME Arrow tool.

## Experimental

### Equipment

Table 1 shows the instrumentation used to perform the experiments.

**Table 1.** Instrumentation used for the experiments.

| Parameter | Value |
|---|---|
| Sample Pretreatment Platform | Agilent PAL3 RTC autosampler with the static headspace, dynamic headspace ITEX, SPME, SPME Arrow tool, Heatex Stirrer, Arrow injection port assembly, fiber conditioning module, and agitator. |
| Static Headspace | 2.5 mL headspace syringe |
| Dynamic Headspace ITEX | 1.3 mL ITEX syringe, Tenax TA 80/100 mesh ITEX trap |
| SPME | DVB/CAR/PDMS sorbent phase (p/n 5191-5874). Before first use, the SPME Arrow was conditioned at 270 °C for 30 min. |
| SPME Arrow | DVB/CAR/PDMS sorbent phase (p/n 5191-5861). Before first use, the SPME Arrow was conditioned at 270 °C for 30 min. |
| GC/TQ Platform | Agilent 8890 GC and 7010B GC/TQ system |

### Reagents and samples

- Ground coffee powder, purchased from a local supermarket
- Milli-Q ultrapure water

### Sample preparation

A 0.25 g sample of coffee powder was placed in an empty 20 mL screw cap vial, then 0.5 mL of ultrapure water was added to the vial. The cap was tightened, and the vial was placed in the PAL3 autosampler sample rack for analysis.

## PAL3 RTC parameters

The analysis parameters of the PAL3 autosampler are shown in Table 2.

**Table 2.** Agilent PAL3 autosampler system parameter settings for coffee analysis.

| Parameter | Value |
|---|---|
| **Headspace** | |
| Sample Volume | 0.5 mL |
| Incubation Time | 10 min |
| Incubation Temperature | 80 °C |
| Syringe Temperature | 100 °C |
| Agitator Speed | 500 rpm |
| **ITEX** | |
| Incubation Time | 10 min |
| Incubation Temperature | 80 °C |
| Syringe Temperature | 100 °C |
| Trap Purge Time | 5 s |
| Trap Extraction Temperature | 40 °C |
| Sample Volume | 500 µL |
| Extraction Strokes | 50 |
| Extraction Volume | 1,000 µL |
| Agitator Speed | 500 rpm |
| **SPME** | |
| Fiber Type | DVB/CWR/PDMS |
| Fiber Conditioning Temperature | 250 °C |
| Preconditioning Time | 5 min |
| Incubation Time | 10 min |
| Incubation Temperature | 80 °C |
| Agitator Speed | 500 rpm |
| Sample Extraction Time | 10 min |
| Sample Desorption Time | 1 min |
| Postconditioning Time | 5 min |
| **SPME Arrow** | |
| Fiber Type | DVB/CWR/PDMS |
| Fiber Conditioning Temperature | 250 °C |
| Preconditioning Time | 10 min |
| Incubation Time | 10 min |
| Incubation Temperature | 80 °C |
| Agitator Speed | 500 rpm |
| Sample Extraction Time | 10 min |
| Sample Desorption Time | 3 min |
| Postconditioning Time | 5 min |

## GC/TQ analysis conditions

The analysis parameters of the GC/TQ instrument are shown in Table 3.

**Table 3.** GC/TQ parameters for coffee analysis.

| Parameter | Value |
|---|---|
| **Gas Chromatograph** | |
| Model | Agilent 8890 GC |
| GC Column | Agilent J&W HP-5ms Ultra Inert column, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI) |
| Column Pneumatics | Constant flow |
| Injector Mode | Pulse split |
| Split Ratio | 5:1 |
| Injector Liner | Agilent Ultra Inert single taper splitless liner (p/n 5190-2292) |
| Inlet Temperature | 250 °C |
| Injector Pulse Pressure | 20 psi until 0.75 min |
| Flow Rate | 1 mL/min |
| Oven Temperature Program | 40 °C for 10 min, 5 °C/min to 100 °C, 20 °C/min to 280 °C, hold 10 min |
| Total Run Time | 41 min |
| Equilibration Time | 3 min |
| **Mass Spectrometer** | |
| Model | Agilent 7010B GC/TQ |
| Ionization Mode | EI, 70 eV |
| Acquisition Mode | MS1 scan |
| Scan Range | 33 to 500 |
| GC Transfer Line Temperature | 280 °C |
| Ion Source Temperature | 230 °C |
| Quad Temperature | 150 °C (MS1), 150 °C (MS2) |
| He Quench Gas | 2.25 mL/min |
| $N_2$ Collision Gas | 1.5 mL/min |

### MassHunter Unknowns Analysis method parameters

Acquired data files were processed using MassHunter Unknowns Analysis software. This tool uses the power of chromatogram deconvolution to extract the compounds, followed by a library search for identification of the compounds.

Unknowns Analysis software is a powerful tool to allow the batch processing of data files for the identification of unknown compounds. Due to the complexity of the samples, coelution is commonly observed. More accurate identification of compounds can be achieved with chromatographic deconvolution, leading to cleaner spectra and a higher library match scores. In addition, blank subtraction can be performed automatically to subtract the compounds that are detected in the blank samples.

The analysis parameters of the Unknowns Analysis tool are shown in Table 4.

**Table 4.** Agilent MassHunter Unknowns Analysis method parameters.

| Parameter | Description |
|---|---|
| Peak Detection | Deconvolution |
| RT Window Size Factor | 25, 50, 100, 200 |
| Library | Wiley 12/NIST 20 |
| Minimum Match Factor | 80 |
| Minimum *m/z* | 33 |
| Library Search Type | Spectral search |
| Perform Blank Subtraction | Yes |

## Results and discussion

Figure 5 shows an overlay chromatogram of the coffee sample analysis using the different sampling techniques. Compared with static headspace, more peaks were detected with dynamic headspace ITEX analysis. The comparison of conventional SPME with the SPME Arrow also indicated enhanced detection of compounds, as observed by the much higher response of the peaks detected. The higher enrichment is due to the larger sorption-phase surfaces and volumes of the SPME Arrow fiber.

Data files that were acquired by the four techniques were processed by MassHunter Unknowns Analysis software with the parameters setting in Table 4. The compounds with a match score of 80 and above with flavor and fragrance relevance are summarized in Table 5 in retention time (RT) order. The aroma information for each of the detected compounds (if available) is also included in the table. All this organoleptic information is obtained from the Good Scents Company Information System.[8] In Table 5, ✓means that the compound was detected by the related technique. The compounds that were uniquely detected by each of the techniques are indicated with ✓ and a blue background.

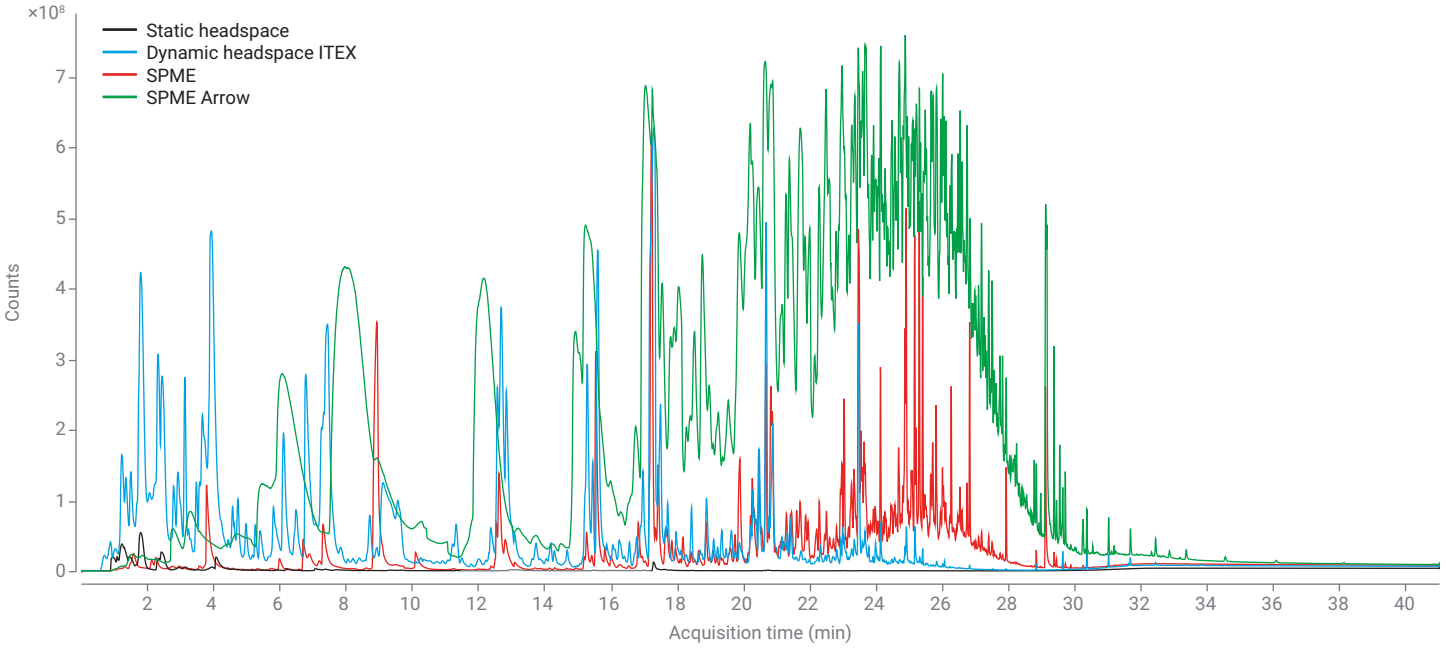


**Figure 5.** Chromatogram of coffee sample analysis using Agilent static headspace, dynamic headspace ITEX, SPME, and SPME Arrow.

**Table 5.** Flavor- and fragrance-related compounds identified in coffee.

| No. | Name | Headspace | ITEX | SPME | SPME Arrow | Aroma Description |
|---|---|---|---|---|---|---|
| 1 | Acetic acid, methyl ester | | ✓ | ✓ | | Green, ethereal, fruity, fresh, rum, whiskey |
| 2 | Propanal, 2-methyl- | | ✓ | | | Fresh, aldehydic, herbal, green, malty |
| 3 | Acetic acid | | | | ✓ | Pungent, sour, fruit, overripe fruit, acetic |
| 4 | Methyl ethyl ketone | ✓ | | | | Ethereal, fruity, green |
| 5 | Furan, 3-methyl- | ✓ | | ✓ | | Natural substances and extractives, no aroma information |
| 6 | 2-Heptanone, 5-methyl- | | | | ✓ | Natural substances and extractives |
| 7 | Butanal, 3-methyl- | ✓ | | ✓ | | Fruity, dry, green, chocolate, nutty, leafy, cocoa |
| 8 | Butanal, 2-methyl- | | ✓ | ✓ | | Cocoa, fruity |
| 9 | 3-Pentanone | | ✓ | | | Ethereal |
| 10 | 2-Pentanone | | ✓ | | | Fruity |
| 11 | Furan, 2-ethyl- | | ✓ | | | Cocoa, bready, malty, coffee, nutty |
| 12 | 2,3-Pentanedione | ✓ | | | | Toasted, buttery, caramellic, marshmallow, molasses |
| 13 | Furan, 2,5-dimethyl- | ✓ | ✓ | | | Meaty, chemical |
| 14 | 2-Vinylfuran | ✓ | ✓ | ✓ | | Phenolic, coffee |
| 15 | 1,3-Dioxolane, 2,4,5-trimethyl- | | ✓ | | | Flavoring agents, no aroma information |
| 16 | Pyrazine | | ✓ | ✓ | | Nutty |
| 17 | 2(5H)-Furanone, 5,5-dimethyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 18 | Methyl vinyl ketone | | ✓ | | | Sweet |
| 19 | 1H-Pyrrole, 2-methyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 20 | Disulfide, dimethyl | ✓ | | | | Sulfurous, cabbage, malty, creamy |
| 21 | Oxazole, 2,4-dimethyl- | ✓ | | | | Natural substances and extractives, no aroma information |
| 22 | Pyridine | ✓ | ✓ | ✓ | ✓ | Sour, fishy |
| 23 | Thiophene, 3-methyl- | ✓ | ✓ | ✓ | | Fatty |
| 24 | 2-Allylfuran | | ✓ | | | Natural substances and extractives, no aroma information |
| 25 | 3-Hexanone | | ✓ | | | Fruity |
| 26 | 2-Methylnon-2-en-4-one | | ✓ | | | Natural substances and extractives, no aroma information |
| 27 | Hexanal | | ✓ | | | Green, woody, vegetable, apple, grassy, citrus, orange, fresh |
| 28 | Thiazole, 2-methyl- | | ✓ | | | Green |
| 29 | 3(2H)-Furanone, dihydro-2-methyl- | ✓ | ✓ | ✓ | ✓ | Nutty, bready |
| 30 | 4-Methylthiazole | | ✓ | ✓ | | Nutty |
| 31 | 1H-Pyrrole, 3-ethyl- | ✓ | | | | Burnt |
| 32 | Pyrazine, methyl- | ✓ | ✓ | ✓ | ✓ | Nutty |
| 33 | 3-Furaldehyde | ✓ | ✓ | ✓ | | Flavoring agents, no aroma information |
| 34 | *p*-Cresol | | ✓ | | | Phenolic |
| 35 | Furan, 2-(methoxymethyl)- | ✓ | ✓ | ✓ | | Coffee |
| 36 | 2-Cyclopenten-1-one, 2-hydroxy- | | ✓ | | | Caramellic |
| 37 | *delta*-2-Dodecenolactone | | ✓ | | | Coconut, lactonic, creamy, fatty |
| 38 | 2-Furanmethanol | ✓ | ✓ | | | Bready, burnt |
| 39 | 3-Furanmethanol | | ✓ | ✓ | | Natural substances and extractives, no aroma information |
| 40 | Thiophene, 2,3-dimethyl- | | ✓ | | | Flavoring agents, no aroma information |

| No. | Name | Headspace | ITEX | SPME | SPME Arrow | Aroma description |
|---|---|---|---|---|---|---|
| 41 | 2-Propanone, 1-(acetyloxy)- | | ✓ | ✓ | ✓ | Fruity, buttery, dairy, nutty |
| 42 | Thiophene, 2,5-dimethyl- | | ✓ | | | Nutty, green, sulfurous |
| 43 | Thiazole, 2,4-dimethyl- | | ✓ | | | Coffee, tea, beefy, barley, roasted |
| 44 | Styrene | | ✓ | | | Almond |
| 45 | 3-Buten-1-ol, 3-methyl-, acetate | | ✓ | | | Fruity |
| 46 | Benzene, 1,3-dimethyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 47 | Thiophene, 3,4-dimethyl- | | ✓ | | | Savory, roasted, onion |
| 48 | 2-Cyclopenten-1-one, 2-methyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 49 | Pyridine, 2-ethyl- | | ✓ | | | Green |
| 50 | Pyrazine, 2,5-dimethyl- | | | ✓ | | Cocoa, chocolate, musty, nutty |
| 51 | Furfuryl formate | | ✓ | | | Ethereal |
| 52 | Pyrazine, 2,6-dimethyl- | | ✓ | ✓ | | Nutty |
| 53 | Ethanone, 1-(2-furanyl)- | | | ✓ | | Sweet, nutty, roasted, baked |
| 54 | Pyrazine, 2,3-dimethyl- | | ✓ | ✓ | | Nutty |
| 55 | Pyrazine, ethyl- | ✓ | ✓ | ✓ | | Nutty |
| 56 | 1-(1-Hydroxycyclohexyl)ethanone | | ✓ | | | Fragrance agents, no aroma information |
| 57 | Pyrazine, ethenyl- | | ✓ | | | Green, burnt, nutty |
| 58 | 2-Cyclopenten-1-one, 2,3-dimethyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 59 | 1H-Pyrrole, 1-butyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 60 | 1-(2-Furyl)-2-propanone | | ✓ | ✓ | ✓ | Fruity |
| 61 | Benzaldehyde | | | ✓ | | Almond, fruity |
| 62 | *p*-Methylanisole | | | ✓ | | Phenolic, nutty |
| 63 | Trisulfide, dimethyl | ✓ | | | | Sulfurous, alliaceous, gassy, savory, meaty, fresh, vegetable |
| 64 | 5-Methyl furfural | ✓ | ✓ | ✓ | | Caramellic |
| 65 | 2-Oxobutyl acetate | | | ✓ | | Natural substances and extractives, no aroma information |
| 66 | Methyl furfuryl thiol | | | ✓ | | Sulfurous, coffee, roasted coffee |
| 67 | Blackberry thiophenone | | | ✓ | | Sulfurous, fruity, berry |
| 68 | Decane, 2,2-dimethyl- | | | ✓ | | Natural substances and extractives, no aroma information |
| 69 | Phenol | | | ✓ | ✓ | Phenolic |
| 70 | Benzofuran | | ✓ | | | Styrene aromatic |
| 71 | Thiazole, 2,4,5-trimethyl- | | ✓ | | | Nutty, chocolate |
| 72 | 3-Thiophenecarboxaldehyde | | ✓ | ✓ | | Natural substances and extractives, no aroma information |
| 73 | 2-Furanmethanol, acetate | ✓ | ✓ | ✓ | | Green, banana skin |
| 74 | Pyrazine, 2-ethyl-6-methyl- | ✓ | ✓ | | | Potato |
| 75 | Pyrazine, trimethyl- | | | ✓ | | Nutty coffee |
| 76 | 1H-Pyrrole-2-carboxaldehyde, 1-methyl- | | | ✓ | | Flavoring agents, no aroma information |
| 77 | Pyrazine, 2-ethyl-3-methyl- | | ✓ | ✓ | | Nutty |
| 78 | 1-Propanone, 1-(2-furanyl)- | | ✓ | ✓ | ✓ | Fruity |
| 79 | Benzoxazole | | ✓ | | | Natural substances and extractives, no aroma information |
| 80 | 2-Acetylthiazole | | ✓ | | | Toasted, corn, chip, popcorn, raw, nutty, bready |
| 81 | Pyrazine, 2-ethenyl-5-methyl- | | ✓ | ✓ | | Coffee |

| No. | Name | Headspace | ITEX | SPME | SPME Arrow | Aroma description |
|---|---|---|---|---|---|---|
| 82 | Butanoic acid, 2-oxo-, methyl ester | | | ✓ | | Natural substances and extractives, no aroma information |
| 83 | D-Limonene | ✓ | ✓ | ✓ | | Citrus |
| 84 | Ethanone, 1-(2-pyridinyl)- | | ✓ | ✓ | | Popcorn |
| 85 | 3,4-Dimethylmaleic Anhydride | | | ✓ | | Natural substances and extractives, no aroma information |
| 86 | Benzeneacetaldehyde | | ✓ | ✓ | ✓ | Green |
| 87 | 2-Cyclohexen-1-one, 4,4-dimethyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 88 | 1-Ethyl-1H-pyrrole-2-carbaldehyde | | ✓ | ✓ | | Roasted, burnt |
| 89 | 3-Carene | | ✓ | | | Citrus |
| 90 | Propanoic acid, anhydride | | | | ✓ | Like acetaldehyde (pungent, fresh, green) |
| 91 | o-Cresol | | ✓ | ✓ | | Phenolic |
| 92 | 2-Cyclopenten-1-one, 2,3,4-trimethyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 93 | Linalool oxide | | ✓ | ✓ | | Earthy, floral, sweet, woody |
| 94 | 2-Acetyl-1-methyl pyrrole | | ✓ | ✓ | | Nutty |
| 95 | Benzofuran, 2,3-dihydro- | | ✓ | ✓ | | Natural substances and extractives, no aroma information |
| 96 | Pyrazine, 3-ethyl-2,5-dimethyl- | | | ✓ | | Hazelnut, nutty |
| 97 | *p*-Cresol | | | ✓ | | Phenolic |
| 98 | Pyrazine, 2,6-diethyl- | | ✓ | | | Nutty |
| 99 | Pyrazine, 2-ethyl-3,5-dimethyl- | ✓ | | | ✓ | Nutty |
| 100 | Furan, 2,2'-methylenebis- | ✓ | ✓ | ✓ | | Rich, toasted, aromatic |
| 101 | Ortho-guaiacol | | | ✓ | | Phenolic, bacon, savory, smoky |
| 102 | Furfuryl propionate | | | ✓ | | Fruity, coffee, green |
| 103 | 2-Ethyl-6-vinylpyrazine | | | ✓ | | Buttery, potato, baked potato |
| 104 | Linalyl formate | | | | ✓ | Citrus, green, herbal |
| 105 | 2-Cyclohexen-1-one, 4,4-dimethyl- | | | ✓ | | Natural substances and extractives, no aroma information |
| 106 | Benzofuran, 2-methyl- | | | | ✓ | Phenolic, burnt |
| 107 | 2(3H)-Furanone, dihydro-5,5-dimethyl- | | | | ✓ | Natural substances and extractives, no aroma information |
| 108 | 2,5-Furandione, 3-ethyl-4-methyl- | | | ✓ | ✓ | Natural substances and extractives, no aroma information |
| 109 | Maltol | | | ✓ | ✓ | Caramellic |
| 110 | 2-Acetyl-4-methylpyridine | | ✓ | ✓ | | Natural substances and extractives, no aroma information |
| 111 | Phenethyl alcohol | | | | ✓ | Floral, bready |
| 112 | 2-Acetyl-5-methyl pyrazine | | | | ✓ | Nutty |
| 113 | 2-Acetyl-3-methylpyrazine | | | ✓ | | Nutty |
| 114 | Ethylcyclopentenolone | | | ✓ | | Sweet, brown, caramellic |
| 115 | Benzyl methyl ketone | | | ✓ | ✓ | Almond |
| 116 | 5H-5-Methyl-6,7-dihydrocyclopentapyrazine | | | ✓ | | Nutty, roasted, grain, coffee |
| 117 | Pyrazine, 2,3-diethyl-5-methyl- | | ✓ | ✓ | | Musty, nutty, toasted, cocoa, hazelnut, potato |
| 118 | Pyrazine, 3,5-diethyl-2-methyl- | | ✓ | | | Nutty |
| 119 | 1-(1-Hydroxycyclohexyl)ethanone | | ✓ | | | Fragrance agents, no aroma information |
| 120 | Benzoic acid, 2,3-dihydroxy- | | | ✓ | | Natural substances and extractives |
| 121 | 2,5-Diethyl-3-methylpyrazine | | ✓ | ✓ | ✓ | Nutty |

| No. | Name | Headspace | ITEX | SPME | SPME Arrow | Aroma description |
|---|---|---|---|---|---|---|
| 122 | Pulegone | | ✓ | | | Minty, sulfurous, fruity |
| 123 | 1H-Pyrrole, 1-(2-furanylmethyl)- | | ✓ | ✓ | ✓ | Plastic, green, waxy, fruity, coffee, vegetable |
| 124 | (R)-Massoia lactone | | | | ✓ | Milky, waxy, creamy, coconut |
| 125 | Methyl salicylate | | ✓ | | | Minty |
| 126 | Dodecane | | ✓ | | | Fragrance agents, no aroma information |
| 127 | Methyl furfuryl disulfide | | ✓ | ✓ | ✓ | Coffee, roasted coffee |
| 128 | Bicyclo[2.2.1]hept-2-ene, 2,3-dimethyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 129 | Furan, 3-phenyl- | | | ✓ | | Natural substances and extractives, no aroma information |
| 130 | Furfuryl isovalerate | | | ✓ | ✓ | Fruity |
| 131 | Para-methyl tetrahydroquinoline | | ✓ | | | Animal, civet, leather, ambergris |
| 132 | Benzoic acid, 2,3-dihydroxy- | | | | ✓ | Natural substances and extractives, no aroma information |
| 133 | 2-Butenal, 4-phenyl- | | ✓ | | | Natural substances and extractives, no aroma information |
| 134 | Guaiacol, 4-ethyl- | | ✓ | ✓ | | Spicy, sweet, vanilla |
| 135 | Indole | | | ✓ | | Pungent, phenolic, floral |
| 136 | Difurfuryl ethyl | | ✓ | ✓ | ✓ | Coffee, nutty |
| 137 | 2-Methoxy-4-vinylphenol | | | ✓ | | Spicy, phenolic |
| 138 | 3,4-Dimethoxy styrene | | ✓ | ✓ | | Green, floral, fruity |
| 139 | (E)-Beta-damascenone | | ✓ | | | Fruity |
| 140 | 1H-Indole, 5-methyl- | | | ✓ | | Natural substances and extractives, no aroma information |
| 141 | Tetradecane | | ✓ | | | Mild, waxy |
| 142 | Cyclamen aldehyde | | | ✓ | | Floral, green |
| 143 | Caffeine | | | ✓ | ✓ | Bitter, astringent, metallic |
| 144 | Methyl palmitate | | | ✓ | ✓ | Oily, waxy, fatty, orris |
| 145 | *n*-Hexadecanoic acid | | | | ✓ | Waxy, creamy, fatty |
| 146 | Ethyl palmitate | | | ✓ | ✓ | Waxy, fruity, creamy, fermented, vanilla, balsamic |

Sulfur-containing compounds contribute a high odor impact on the taste of coffee even though trace amounts are present. The low concentration levels of these compounds makes their detection difficult. With the combination of the four techniques, 14 sulfur-containing compounds were detected: disulfide dimethyl, thiophene, 3-methyl-, thiazole, 2-methyl-, 4-methylthiazole, thiophene, 2,3-dimethyl-, thiazole, 2,4-dimethyl-, thiophene, 3,4-dimethyl-, trisulfide, dimethyl, methyl furfuryl thiol, blackberry thiophenone, thiazole, 2,4,5-trimethyl-, 3-thiophenecarboxaldehyde, 2-acetylthiazole, and methyl furfuryl disulfide.

The representative aroma compounds of coffee including different kinds of pyrazine (nutty flavor), maltol (caramel flavor), and caffeine (bitter and astringent flavor) were also detected. With this work, 146 compounds with flavor and fragrance relevance were identified in the coffee sample acquired using the combination of the four sample introduction techniques.

It was observed that there were more compounds reported with SPME (77) compared to SPME Arrow (31). This was due to saturation of the detector in the SPME Arrow setup as it had larger sorption-phase surfaces and volumes compared to SPME fiber. The sample amount and acquisition parameters (including split ratio and MS gain factor) were kept the same for comparison across the four techniques in this study. The sample amount and acquisition parameters could be optimized to prevent the saturation of the detector for specific SPME Arrow analysis.

For complex chromatograms with a high possibility of coelution of adjacent peaks, deconvolution helps to provide a clean component spectrum, improving library match quality. In Table 6, the component pyrazine, methyl- with an RT of 6.698 minutes was chosen to demonstrate the value of deconvolution as an important step in data processing (as shown in Figure 6).

**Table 6.** Components table with compound identification and library match factor (SPME ).

Components

| Component RT | Compound Name | Match Factor | Formula | CAS# |
|---|---|---|---|---|
| 3.780 | Pyridine | 97.8 | C5H5N | 110-86-1 |
| 4.571 | Thiophene, 3-methyl- | 87.7 | C5H6S | 616-44-4 |
| 5.982 | 3(2H)-Furanone, dihy... | 89.7 | C5H8O2 | 3188-00-9 |
| 6.558 | 4-Methylthiazole | 85.4 | C4H5NS | 693-95-8 |
| 6.698 | Pyrazine, methyl- | 91.1 | C5H6N2 | 109-08-0 |
| 7.174 | Furan, 2-(methoxyme... | 95.0 | C6H8O2 | 13679-46-4 |
| 7.299 | 3-Furaldehyde | 89.1 | C5H4O2 | 498-60-2 |
| 8.921 | 3-Furanmethanol | 92.5 | C5H6O2 | 4412-91-3 |
| 9.511 | 2H-Pentaleno[1,6-bc]f... | 89.7 | C9H10O2 | 127268-74-0 |

With MassHunter Unknowns Analysis software, a clean deconvoluted spectrum for the peak at RT 6.698 minutes (as shown in Figure 6A) was obtained. Due to the coelution of the adjacent peaks, there were ions (206.9, 190.8, 162.8, 132.9, and 107.9) that did not belong to the compound present in the raw mass spectrum (Figure 6C). The deconvoluted spectrum (Figure 6A) enabled the compound to be identified with a good match score of 91.1 (as shown in Table 8) against the library spectrum (Figure 6B).

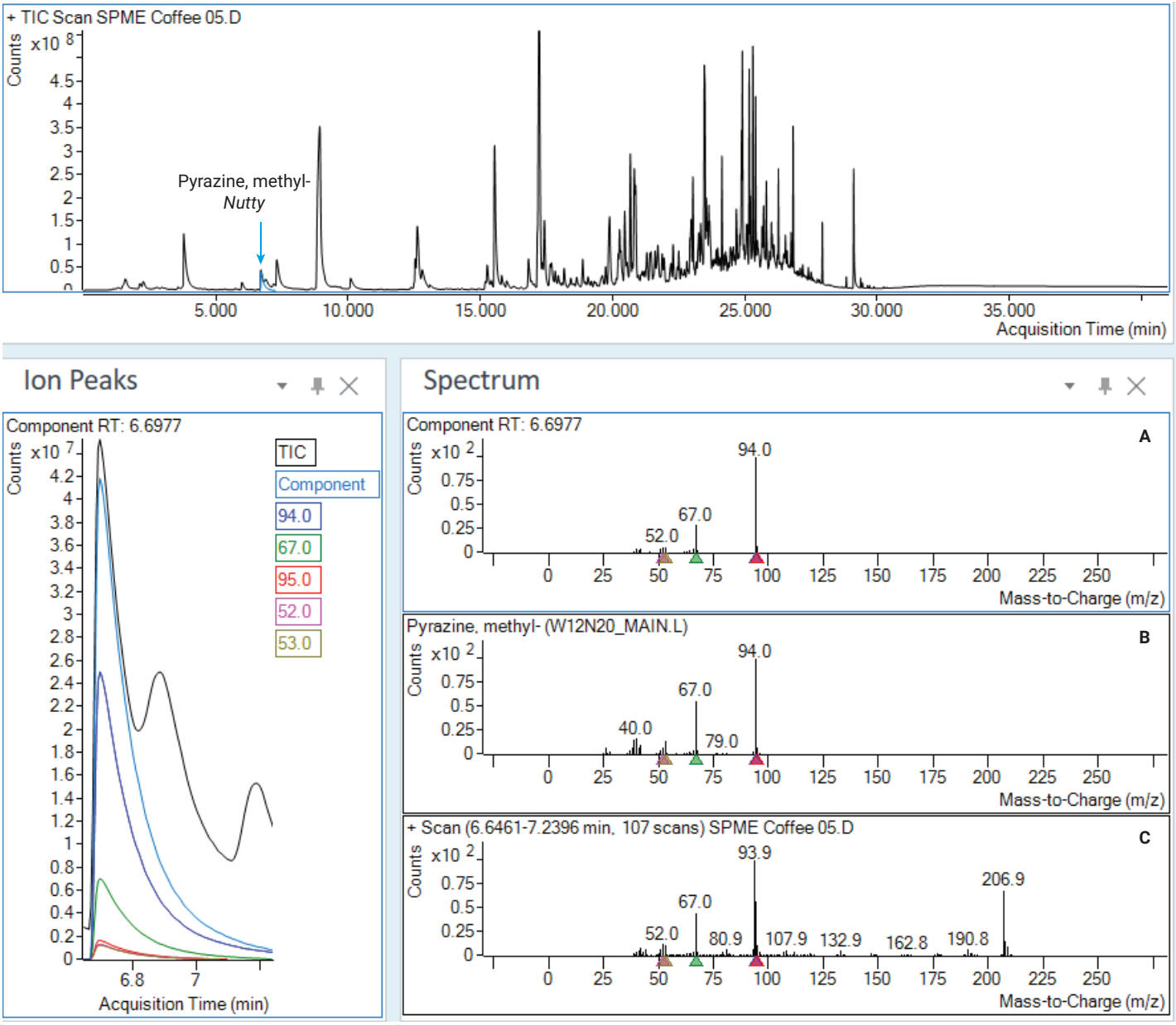


**Figure 6.** Agilent MassHunter Unknowns Analysis software uses a deconvolution algorithm to separate coeluting compounds. The compound deconvoluted spectrum (A), library spectrum (B), and raw spectrum (C) are displayed.

Components with trace concentrations can be difficult to detect and identify from complex matrix interference. Performing deconvolution of the data can help with the detection and identification of such trace-level components. In Table 7, the component trisulfide, dimethyl with a retention time of 15.622 minutes is a trace compound (as shown in Figure 7).

**Table 7.** Components table with compound identification and library match factor (static headspace ).

Components

| Component RT | Compound Name | Match Factor | Formula | CAS# |
|---|---|---|---|---|
| 7.389 | Furan, 2-(methoxyme... | 89.5 | C6H8O2 | 13679-46-4 |
| 7.701 | 3-Furaldehyde | 84.0 | C5H4O2 | 498-60-2 |
| 9.373 | 2-Furanmethanol | 82.9 | C5H6O2 | 98-00-0 |
| 13.121 | Pyrazine, ethyl- | 90.7 | C6H8N2 | 13925-00-3 |
| 15.622 | Trisulfide, dimethyl | 86.3 | C2H6S3 | 3658-80-8 |
| 15.894 | 5-Methyl furfural | 86.6 | C6H6O2 | 620-02-0 |
| 17.275 | 2-Furanmethanol, ace... | 94.1 | C7H8O3 | 623-17-6 |
| 17.308 | Pyrazine, 2-ethyl-6-m... | 83.0 | C7H10N2 | 13925-03-6 |
| 18.438 | D-Limonene | 82.2 | C10H16 | 5989-27-5 |

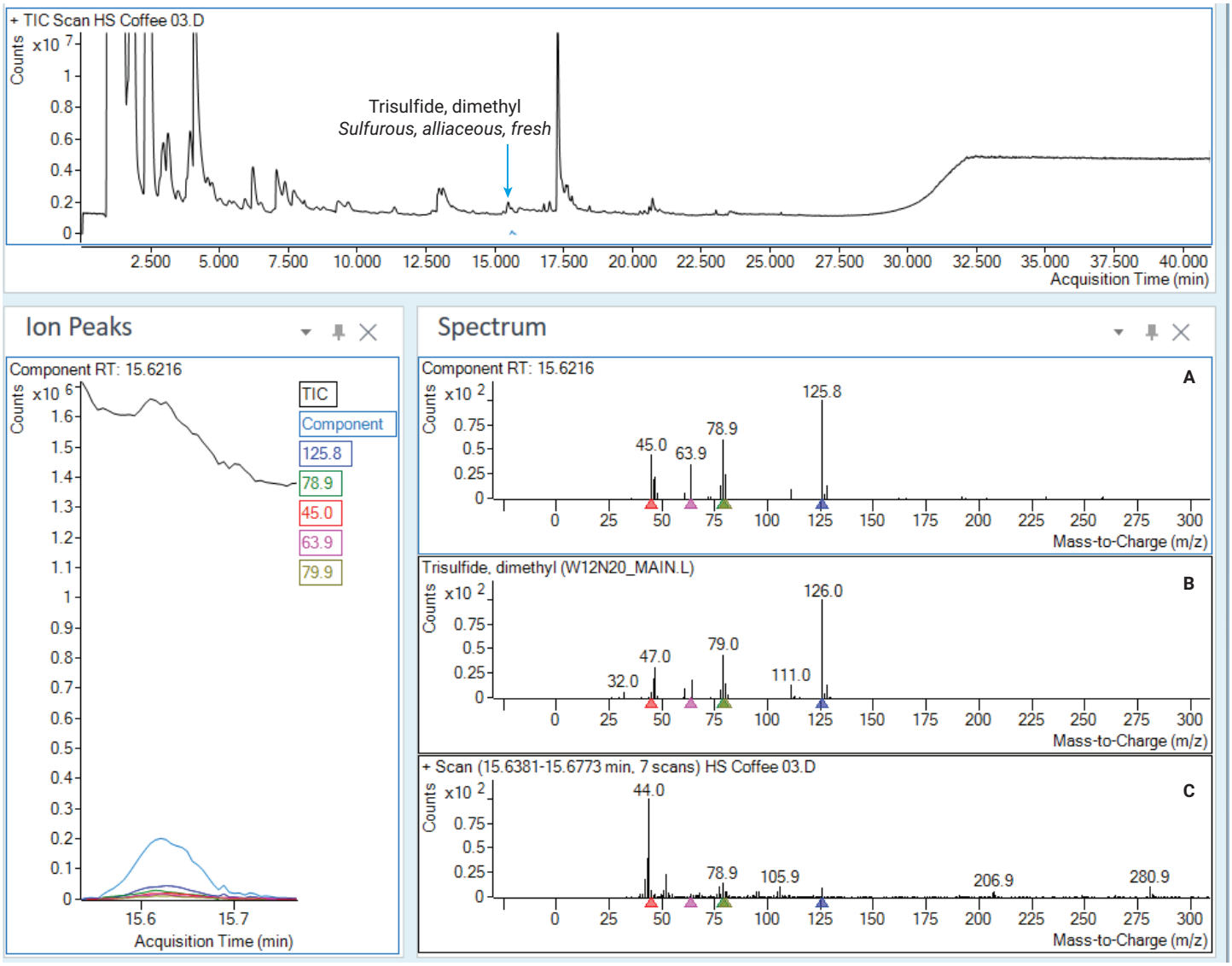


**Figure 7.** Agilent MassHunter Unknowns Analysis software uses a deconvolution algorithm to separate coeluting compounds. The compound deconvoluted spectrum (A), library spectrum (B), and raw spectrum (C) are displayed.

For the very small peak at 15.622 minutes in Figure 7, the carbon dioxide (a component of air, with molecular ion of 44) background interference (Figure 7C) was eliminated through deconvolution processing to generate a clean spectrum (Figure 7A). It is identified as a sulfur compound, trisulfide dimethyl (library match score of 86.3, as shown in Table 7), which is associated with being alliaceous in taste or smell.

## Conclusion

This application note describes full coffee aroma profiling using the Agilent PAL3 RTC autosampler, 8890 GC, 7010B triple quadrupole GC/MS system, and MassHunter Unknowns Analysis software. Four Agilent sampling tools, including the static headspace tool, dynamic headspace ITEX tool, SPME tool, and SPME Arrow tool were used. In this application note, 146 compounds of flavor and fragrance relevance were detected with the four sample introduction techniques. Among these 146 compounds, 6, 48, 27, and 11 compounds were uniquely detected using headspace, dynamic headspace ITEX, SPME, and SPME Arrow, respectively. The rest of the compounds were detected using at least two of the sample introduction techniques. These four techniques are complementary in nature and can be used to uncover the complete volatile aroma profile of coffee.

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