Application Note

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# Highly Selective GC/TQ Analysis of 57 Allergens in Cosmetic Products

## Featuring the Agilent 8890 GC and Agilent 7000E GC/TQ systems


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## Abstract

With the expansion of the list of potential allergens, cosmetics manufacturers need to identify, quantify, and label the contents in their products. Cosmetic products are complex mixtures, which can be challenging to analyze due to the presence of many compounds that could cause chromatographic and spectral interferences.

In this application note, an Agilent 8890 GC with a dual-column setup and an Agilent 7000E GC/TQ provide high selectivity for the analysis of 57 allergen fragrance substances and their relevant isomers in cosmetic products. This GC/TQ method is quickly and easily transferred from an existing gas chromatograph/mass selective detector (GC/MSD) method using Agilent MassHunter Optimizer software. The high selectivity and reliable quantitation of the GC/TQ method increase confidence in data by reducing uncertainty caused by coelution and therefore the risk of false positives and negatives.

## Introduction

Allergens include diverse compounds, both synthetic and natural, that cause allergic reactions in sensitive people. Allergens can be found in many cosmetic products, including shower gels, soaps, shampoos, creams, makeup, etc. These compounds are often not intentionally added to cosmetics during manufacturing, but are substances contained in the base ingredients that make up the final product. This work focuses on a method to determine allergens commonly found in fragrance substances.

Regulating agencies around the world have—and are frequently updating—regulations around allergens in consumer products, like cosmetics and fragrances. In 2012, the European Scientific Committee on Consumer Safety (SCCS) identified 87 fragrance substances that can cause allergies in humans. So, in 2023, the European Commission published Regulation 2023/1545, amending Regulation (EC) Number 1223/2009 of the European Parliament and of the Council, concerning the labeling of allergenic substances in cosmetic products.[1] The new regulation expanded the historical list of 26 allergens to 87 compounds.

These regulations are not limited to Europe. The Modernization of Cosmetics Regulation Act of 2022 (MoCRA) is the most significant expansion of the U.S. Food and Drug Administration's authority to regulate cosmetics since the Federal Food, Drug, and Cosmetic (FD&C) Act was passed in 1938.[2] These updated laws and regulations help to ensure the safety of cosmetic products. To determine the presence of allergens in cosmetics, a sensitive and selective analytical technique is required, such as analysis by GC/TQ.

## Experimental

An 8890 GC configured with an 7000E GC/TQ controlled by MassHunter Workstation software was used for this work. To analyze compounds in complex matrices, such as cosmetic products, it is beneficial to have two columns of different polarity to confirm results. In this case, an Agilent J&W DB-WAX Ultra Inert GC column was used for the polar stationary phase and an Agilent J&W DB-5ms Ultra Inert GC column was used for the apolar stationary phase. An inert compact splitter (part number G3181-60500) with flexible gold-plated ferrules (part number G2855-28501) configured with 1.5 m of deactivated fused silica tubing (part number CP801810) allows both columns to be connected simultaneously without needing to stop or vent the mass spectrometer, maximizing instrument uptime.

An illustration of the instrument configuration is shown in Figure 1. Both columns used the same analytical method, which is detailed in Tables 1 and 2. The calibration solutions used for analysis were prepared through serial dilution using fragrance allergen mix A1 and A2 from Millipore Sigma (product numbers 89131 and 16558).

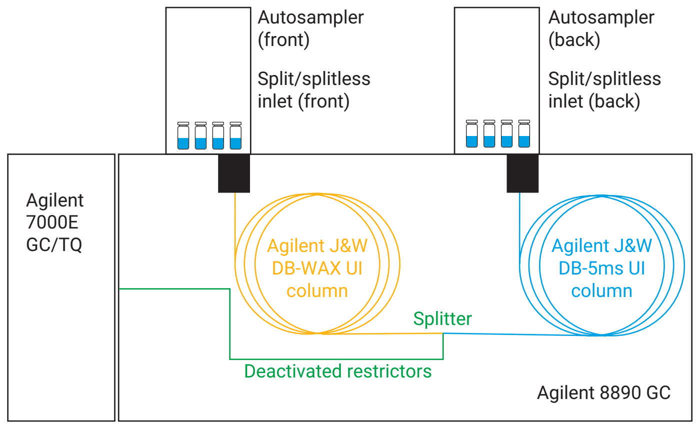


**Figure 1.** GC/TQ instrument setup for the dual-column analysis of allergens.

**Table 1.** Method parameters for the analysis of allergens on an Agilent 8890 GC with an Agilent 7000E GC/TQ.

| | Polar Method | Apolar Method |
|---|---|---|
| Injection Volume | 1 µL | |
| Injection Mode | Split (1:10) | |
| Liner | Agilent Ultra Inert liner (p/n 5190-2295) | |
| Column | Agilent J&W DB-WAX Ultra Inert GC column, 20 m × 0.18 mm, 0.18 µm (p/n 121-7022UI) | Agilent J&W DB-5ms Ultra Inert GC column, 20 m × 0.18 mm, 0.18 µm (p/n 121-5522UI) |
| Column Flow | Constant flow: 1 mL/min (0.5 mL/min when not in use) | |
| Oven Program | Hold 50 °C for 0.5 min; ramp to 200 °C at 7 °C/min; ramp to 240 °C at 25 °C/min; hold 240 °C for 10 min | |
| MS Transfer Line | 240 °C | |
| Source Temperature | 250 °C | |
| Quadrupole Temperature | 150 °C | |
| Acquisition Mode | MRM/Scan (MS2) | |

**Table 2.** GC/TQ multiple reaction monitoring (MRM) transitions for the analysis of allergens on an Agilent 7000E GC/TQ.
(Continued on next page).

| Compound Number | Compound Name | CAS Registry Number | Transition 1 (*m/z*) | Transition 2 (*m/z*) | Transition 3 (*m/z*) |
|---|---|---|---|---|---|
| 1 | Pinene <alpha-> | 80-56-8 | 121.0 → 77.0 | 93.0 → 77.0 | 136.0 → 93.0 |
| 2 | Pinene <beta-> | 127-91-3 | 121.0 → 93.0 | 93.0 → 77.0 | 136.0 → 93.0 |
| 3 | Terpinene <alpha-> | 99-86-5 | 121.0 → 93.0 | 93.0 → 77.0 | 136.0 → 93.0 |
| 4 | Limonene | 138-86-3 | 136.0 → 94.0 | 68.0 → 67.0 | 93.0 → 77.0 |
| 5 | Terpinolene | 586-62-9 | 121.0 → 93.0 | 93.0 → 77.0 | 136.0 → 93.0 |
| 6 | Camphor | 76-22-2 | 152.0 → 108.0 | 108.0 → 93.1 | 95.0 → 67.0 |
| 7 | Benzaldehyde | 100-52-7 | 106.0 → 105.0 | 105.0 → 77.0 | 77.0 → 51.0 |
| 8 | Linalol | 78-70-6 | 121.0 → 93.0 | 93.0 → 77.0 | 136.0 → 93.1 |
| 9 | Linalyl acetate | 115-95-7 | 121.0 → 93.0 | 93.0 → 77.0 | 136.0 → 93.1 |
| 10 | Caryophyllene <(B)-> | 87-44-5 | 189.0 → 91.1 | 133.0 → 105.1 | 93.0 → 77.0 |
| 11 | Menthol | 89-78-1 | 123.0 → 81.0 | 95.0 → 67.0 | 138.0 → 95.0 |
| 12 | Oct-2-ynoate <methyl-> (Folione) | 111-12-6 | 123.0 → 67.0 | 95.0 → 67.0 | 139.0 → 77.0 |
| 13 | Salicylaldehyde | 90-02-8 | 122.0 → 121.0 | 121.0 → 65.0 | 93.0 → 65.0 |
| 14 | Terpineol <alpha-> | 98-55-5 | 121.0 → 93.0 | 93.0 → 77.0 | 136.0 → 93.0 |
| 15-1 | Neral | 106-26-3 | 109.0 → 81.0 | 69.0 → 41.1 | 134.0 → 119.0 |
| 15-2 | Geranial | 141-27-5 | 137.0 → 43.0 | 84.0 → 83.0 | 152.0 → 137.0 |
| 16 | Carvone | 99-49-0 | 108.0 → 93.0 | 82.0 → 39.1 | 150.0 → 108.0 |
| 17 | Geranyl acetate | 105-87-3 | 121.0 → 76.9 | 93.0 → 77.0 | 136.0 → 121.0 |
| 18 | Damascone <delta-> | 57378-68-4 | 123.0 → 81.0 | 69.0 → 41.1 | 192.0 → 135.0 |
| 19 | Salicylate <methyl-> | 119-36-8 | 152.0 → 92.0 | 120.0 → 92.0 | 92.0 → 63.0 |
| 20 | Acetate DMBC (dimethylbenzylcarbinyl) | 151-05-3 | 117.0 → 91.0 | 132.0 → 117.0 | 91.0 → 65.0 |
| 21 | Damascone <alpha-, *trans*-> | 43052-87-5 | 123.0 → 81.0 | 69.0 → 41.1 | 192.0 → 135.0 |
| 22 | Citronellol | 106-22-9 | 81.0 → 53.1 | 81.0 → 41.1 | 138.0 → 95.0 |
| 23 | Damascone <(E)-beta-> | 23726-91-2 | 123.0 → 81.0 | 192.0 → 177.1 | 177.0 → 77.0 |
| 24 | Damascenone <(E)-, beta-> | 23696-85-7 | 121.0 → 77.0 | 69.0 → 41.1 | 190.1 → 121.0 |
| 25 | Anethole <(E)-> | 104-46-1 | 117.0 → 91.0 | 147.0 → 91.0 | 148.0 → 77.0 |
| 26 | Ionone <alpha-, isomethyl-> | 127-51-5 | 135.0 → 91.0 | 107.0 → 91.0 | 150.0 → 135.0 |
| 27 | Geraniol | 106-24-1 | 123.0 → 43.1 | 93.0 → 77.0 | 123.0 → 77.0 |
| 28 | Benzyl alcohol | 100-51-6 | 107.0 → 79.0 | 108.0 → 79.0 | 79.0 → 77.0 |
| 29-1 | Ebanol 1 | 67801-20-1 | 149.0 → 107.1 | 121.0 → 77.0 | 164.0 → 149.1 |
| 29-2 | Ebanol 2 | 67801-20-1 | 149.0 → 107.0 | 121.0 → 77.0 | 164.0 → 149.1 |
| 30 | Hydroxycitronellal | 107-75-5 | 111.0 → 69.0 | 95.0 → 67.0 | 139.0 → 43.0 |
| 31 | Cinnamaldehyde <(E)-> | 104-55-2 | 103.0 → 77.0 | 131.0 → 77.0 | 132.0 → 131.0 |
| 32 | Butylphenyl methylpropional (lilial) | 80-54-6 | 147.0 → 117.0 | 189.0 → 91.0 | 204.0 → 189.0 |
| 33-1 | Iso E Super <beta-> | 54464-57-2 | 119.0 → 91.0 | 191.0 → 109.1 | 121.0 → 93.0 |
| 33-2 | Iso E Super <alpha-> | 68155-66-8 | 135.0 → 107.0 | 69.0 → 41.1 | 191.0 → 121.1 |
| 34 | Salicylate <amyl-> | 2050-08-0 | 138.0 → 92.0 | 120.0 → 92.0 | 208.0 → 120.0 |
| 33-3 | Iso E super <gamma-> | 68155-67-9 | 150.0 → 135.0 | 135.0 → 107.0 | 191.0 → 121.1 |
| 35 | Trimethyl-benzenepropanol (majantol) | 103694-68-4 | 105.0 → 77.0 | 106.0 → 91.0 | 178.0 → 106.0 |
| 36 | Eugenol | 97-53-0 | 131.0 → 77.0 | 164.0 → 77.0 | 149.0 → 77.0 |
| 37 | Vertofix coeur | 32388-55-9 | 203.0 → 43.1 | 231.0 → 43.1 | 246.0 → 43.1 |
| 38 | Cinnamaldehyde <amyl-> | 122-40-7 | 133.0 → 55.0 | 129.0 → 128.0 | 201.0 → 145.0 |
| 39 | Eugenyl acetate | 93-28-7 | 131.0 → 77.0 | 164.0 → 77.0 | 149.0 → 77.0 |
| 40 | Anisyl alcohol <para-> | 105-13-5 | 137.0 → 77.0 | 109.0 → 77.0 | 138.0 → 77.0 |
| 41 | Cinnamyl alcohol <(E)-> | 104-54-1 | 92.0 → 39.1 | 92.0 → 91.0 | 115.0 → 89.0 |

**Table 2.** GC/TQ multiple reaction monitoring (MRM) transitions for the analysis of allergens on an Agilent 7000E GC/TQ. (Continued from previous page).

| Compound Number | Compound Name | CAS Registry Number | Transition 1 (*m/z*) | Transition 2 (*m/z*) | Transition 3 (*m/z*) |
|---|---|---|---|---|---|
| 42-1 | Galaxolide 1 | 1222-05-05 | 213.0 → 171.0 | 243.0 → 213.1 | 258.0 → 243.1 |
| 42-2 | Galaxolide 2 | 1222-05-05 | 213.0 → 171.0 | 243.0 → 213.1 | 258.0 → 243.1 |
| 43-1 | alpha-Santalol | 115-71-9 | 122.0 → 94.0 | 94.0 → 79.0 | 93.0 → 77.0 |
| 43-2 | beta-Santalol | 77-42-9 | 122.0 → 94.0 | 94.0 → 79.0 | 93.0 → 77.0 |
| 44 | Farnesol | 4602-84-0 | 133.0 → 105.0 | 69.0 → 41.1 | 93.0 → 77.0 |
| 45 | Isoeugenol | 97-54-1 | 131.0 → 77.0 | 164.0 → 77.0 | 149.0 → 77.0 |
| 46 | Cinnamaldehyde <alpha-hexyl-> (jasmonal) | 101-86-0 | 117.0 → 91.0 | 129.0 → 128.0 | 216.0 → 129.0 |
| 47 | Hexadecanolact-16-one | 109-29-5 | 193.0 → 81.1 | 97.0 → 55.0 | 236.0 → 80.9 |
| 48 | Phthalide <3-propylidene-> *cis* and *trans* | 17369-59-4 | 159.0 → 131.0 | 159.0 → 103.0 | 146.0 → 105.0 |
| 49 | Isoeugenyl acetate | 93-29-8 | 149.0 → 77.0 | 164.0 → 77.0 | 165.0 → 77.9 |
| 50 | Coumarin | 91-64-5 | 118.0 → 89.0 | 146.0 → 118.0 | 90.0 → 89.0 |
| 51-1 | Lyral 1 | 31906-04-4 | 136.0 → 79.0 | 93.0 → 77.0 | 192.0 → 91.0 |
| 51-2 | Lyral 2 | 31906-04-4 | 136.0 → 79.0 | 93.0 → 77.0 | 192.0 → 91.0 |
| 52 | Amyl cinnamyl alcohol | 101-85-9 | 133.0 → 115.0 | 133.0 → 55.0 | 133.0 → 77.0 |
| 53 | Vanillin | 121-33-5 | 123.0 → 52.0 | 152.0 → 151.0 | 151.0 → 52.0 |
| 54 | Benzyl benzoate | 120-51-4 | 194.0 → 165.0 | 105.0 → 77.0 | 212.0 → 105.0 |
| 55 | Benzyl salicylate | 118-58-1 | 228.0 → 91.0 | 91.0 → 65.0 | 228.0 → 65.0 |
| 56 | Sclareol | 515-03-7 | 191.1 → 94.9 | 177.0 → 121.1 | 257.1 → 119.2 |
| 57 | Cinnamate <benzyl-> | 103-41-3 | 192.0 → 191.0 | 131.0 → 77.0 | 237.8 → 192.0 |

## Results and discussion

Previous work demonstrates the analysis of 57 suspected allergens by a GC/MSD in selected ion monitoring (SIM) mode.[3] When using a GC/MSD in SIM mode, care must be taken during method development to ensure that all target peaks can be adequately resolved, and that no interference of the SIM ions used for quantification takes place. For complex matrices with many target analytes, sufficient chromatographic resolution can be challenging using a GC/MSD. However, transferring such a complex analysis to a triple quadrupole GC/MS (GC/TQ) can provide significant gains in selectivity.

GC/TQ instruments offer multiple reaction monitoring (MRM) mode for data acquisition. MRM mode is used to measure ions resulting from collision-induced dissociation (CID). The nature of CID reactions depends on molecular structure as well as mass and allows for significant improvements in selectivity. With the added benefit of simultaneous acquisition in MRM/Scan mode, users can ensure that they not only accurately analyze their targets, but they can also ensure a more complete sample characterization with a full scan.

Transferring a GC/MSD method to a GC/TQ method is quick and easy with Agilent MassHunter Optimizer software. The SIM ions already provided by the GC/MSD method can be set as the precursor ions for the GC/TQ in MassHunter Optimizer, then the software can be set up to run automatically to determine the optimal MRM transitions for all target analytes for the GC/TQ method. All GC method parameters and GC/MS source parameters can remain the same for both instrument methods.

Using the method optimized by MassHunter Optimizer software, the separation of 57 allergens on two Agilent J&W GC columns is demonstrated in Figure 2. Adequate resolution is achieved for all target compounds, demonstrating the improved selectivity provided by the 7000E GC/TQ system. Six representative calibration curves and 1 mg/kg allergen standard analyses are shown in Figure 3. Calibration curves plot the response from allergen standards ranging in concentration from 1 to 100 mg/kg. Curve fits are quadratic, ignoring the origin, with 1/x weighting and all correlation coefficients ($R^2$) > 0.997, indicating reliable quantitation for this GC/TQ method on both J&W GC columns.

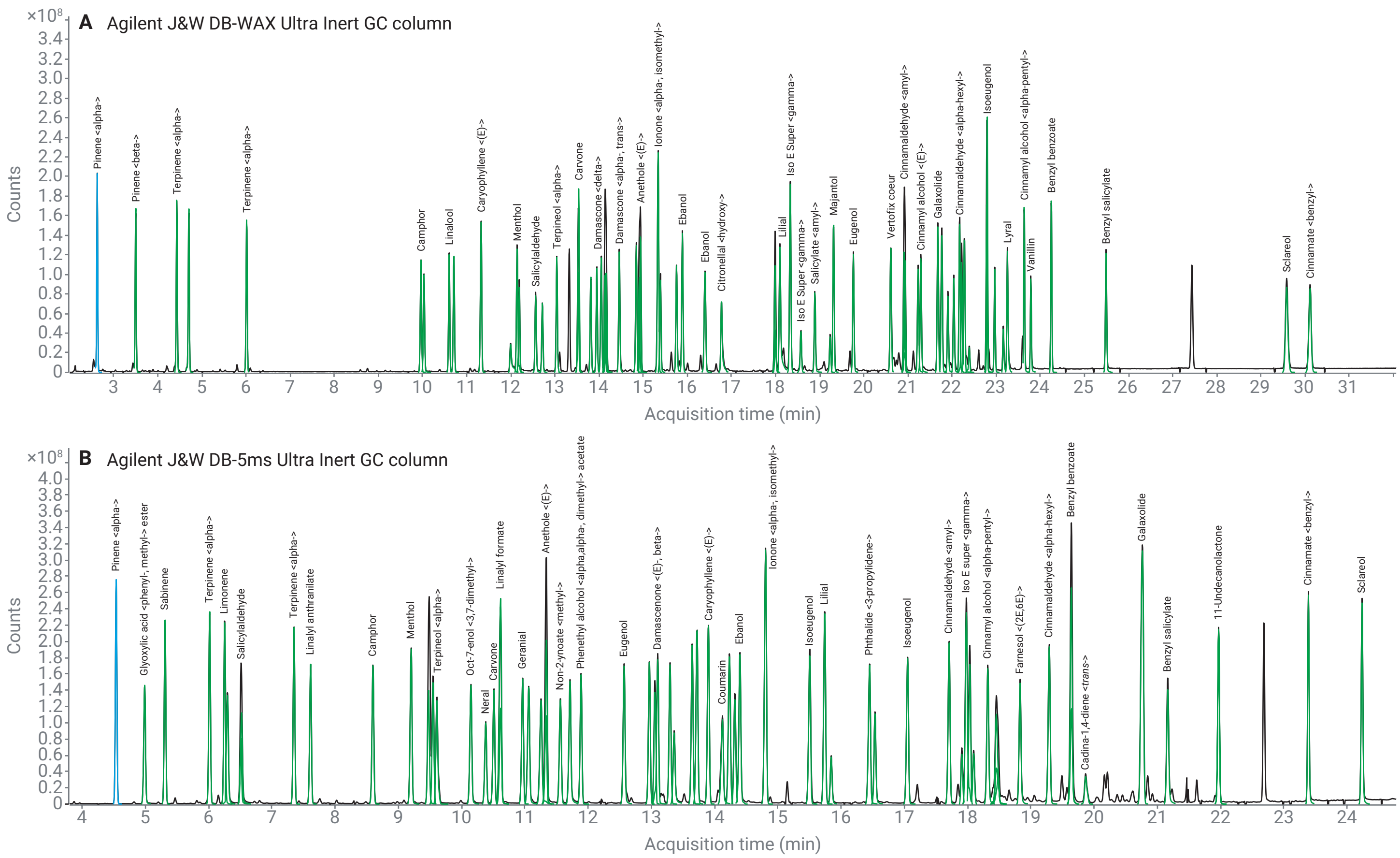


**Figure 2.** Chromatographic separation of 100 mg/kg allergens standard on two Agilent J&W GC columns—(A) a polar column and (B) an apolar column.

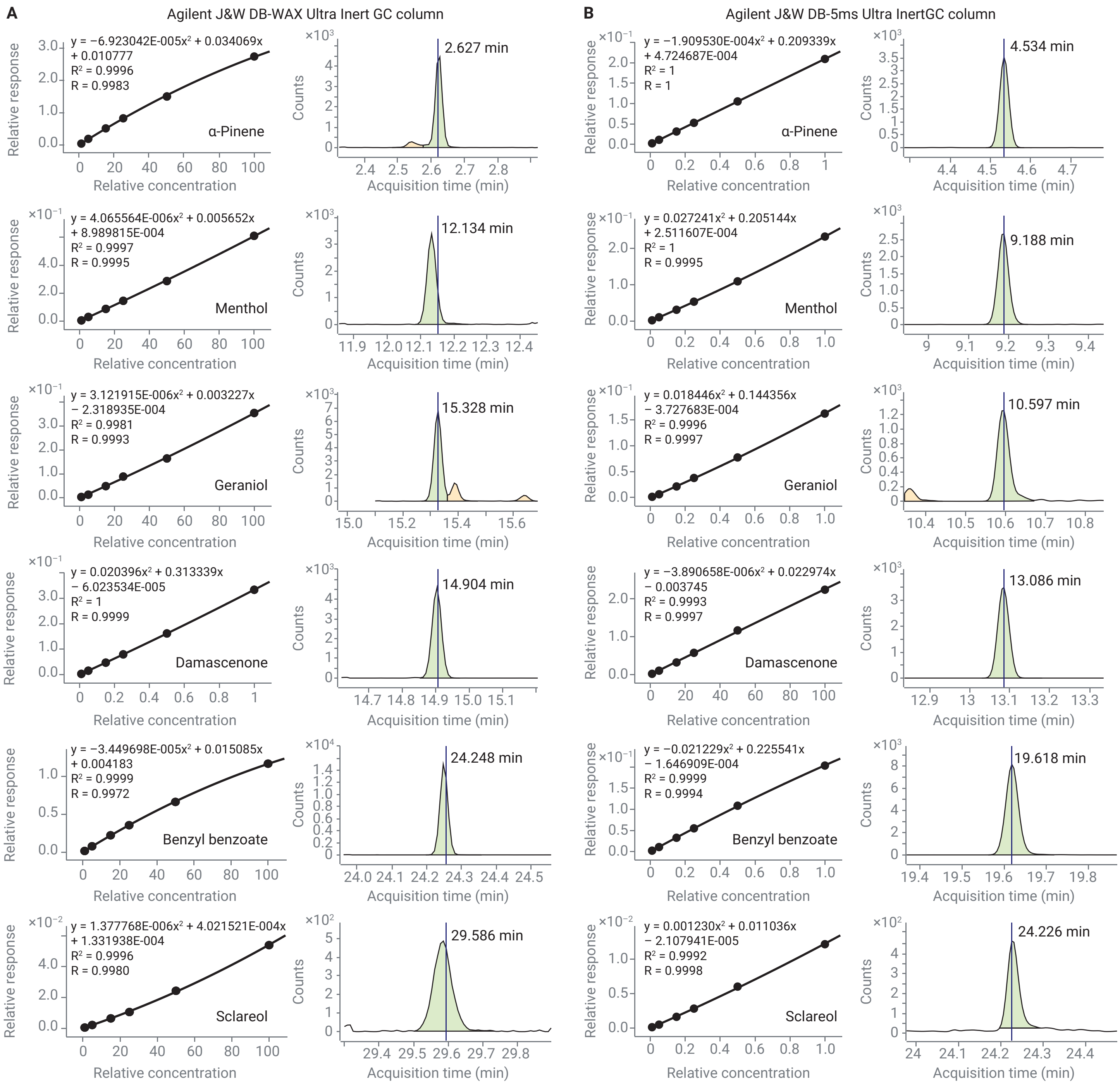


**Figure 3.** Example calibration curve and 1 mg/kg standard chromatogram for six allergens on two Agilent J&W GC columns with detection by an Agilent 7000E GC/TQ.

## Conclusion

Migrating an established GC/MSD-based allergen analysis to GC/TQ is quick and easy, and it improves method selectivity. The same chromatographic and MS source method parameters, columns, and consumables can be used for the new GC/TQ method. For the new MS acquisition method, Agilent MassHunter Optimizer software can be automated to determine optimal MRM transitions for the GC/TQ method using the SIM ions from the original GC/MSD method. The resulting improved GC/TQ method selectivity increases confidence in data, reducing the risk of false positives and negatives for coeluting target allergens. The new GC/TQ method in this work provides reliable quantitation for the analysis of 57 allergen fragrance substances and their relevant isomers in cosmetic products using an Agilent 8890 GC with an Agilent 7000E GC/TQ.

## References


1. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. *Official Journal of the European Union*, **2009**.
2. Modernization of Cosmetics Regulation Act of 2022 (MoCRA). U.S. Food and Drug Administration, **2023**.
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