

# Quality Control of Fragrance Samples by GC-FID: Method Transfer from the Agilent 7890 GC to the Agilent Intuvo 9000 GC

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## Abstract

Quality control of essential oils, fragrances, and perfumes is typically performed by GC-FID analysis. Important GC performance criteria are resolution, stability of retention times and indices, and sample throughput. This Application Note describes the transfer of a reference method developed on an Agilent 7890 GC to the Agilent Intuvo 9000 GC, with the comparison of data obtained on typical fragrance and essential oil samples. It demonstrates that data quality is maintained on the Intuvo 9000 GC, while sample throughput can be increased using shorter narrow bore columns.

## Introduction

Quality control (QC) in the flavor and fragrance industries is typically done by gas chromatography (GC).<sup>1,2</sup> For fingerprint analysis and R&D, GC with mass spectrometry (MS) is frequently applied. In a QC environment, however, GC-FID analysis is the method of choice.<sup>3</sup> The GC-FID profile obtained for a production sample of an essential oil, a perfume, or a fragrance oil is compared to a reference profile of an approved sample of known composition. Both relative sample composition (area %) and the presence of contaminants or impurities are verified. Falsification and origin authenticity can be controlled from the presence, absence, or relative concentrations of marker compounds.

As fragrances consist of mixtures of essential oils and synthetic fragrance compounds, these products often contain more than 100 chemical constituents. Therefore, high-resolution GC methods are required for accurate profiling. Chromatographic resolution and retention time or retention index (RI) reproducibility are the most important system suitability criteria.<sup>4</sup> In a QA/QC environment, productivity and sample throughput are also important, and several approaches are used to reduce GC run times. These include the application of short narrow bore columns, hydrogen carrier gas, and fast temperature programming. However, adequate chromatographic resolution should be preserved.<sup>5</sup>

Fragrances and essential oils are also characterized by a wide range of concentrations of the individual solutes, with some constituents at a high percent level (1 to 50%) and other solutes at a 0.01 to 0.1% level. Since these trace compounds can be important for fragrance quality, the high-resolution GC method should also offer a wide dynamic range, and the selected GC column must have sufficient solute capacity.

High-performance GC-FID methods for QA/QC of fragrances, perfumes, and essential oils are therefore based on a delicate balance between chromatographic resolution, speed of analysis (sample throughput), and solute capacity (sensitivity). This can be achieved using state-of-the-art GC column technology using 150 to 250  $\mu\text{m}$  id columns with hydrogen as carrier gas. In combination with state-of-the-art GC instrumentation, allowing fast heating and cooling cycles enables labs to increase productivity while preserving method performance.

This Application Note demonstrates the GC analysis of some typical fragrance and essential oil samples using the Intuvo 9000 GC. The samples were first analyzed using a standard method on a 7890 GC, and methods were transferred to the Intuvo 9000 GC. In addition, method translation was applied to increase analysis speed by a factor of 2 to 4, taking advantage of the fast heating capability of the instrument.

## Experimental

### Sample preparation

A fragrance oil was obtained from a household product manufacturer, and two essential oil samples were acquired from a local drugstore. One commercial perfume sample was acquired from a perfume store. Samples were diluted in acetone, typically at concentrations between 25 to 100 mg/mL, depending on the initial dilution. The fragrance oil, used in the production of household products such as detergents and surface cleaners, was of a high complexity, with >100 constituents. For GC analysis, this oil was diluted 40-fold (2.5% in acetone). The commercial perfume sample was of medium complexity and diluted 10-fold in acetone.

The essential oil samples were lemon oil (origin: California, USA), mainly consisting of limonene, and a complex sandalwood oil, mainly consisting of sesquiterpenes. The essential oil samples were diluted to 5% in acetone.

These samples were only used for instrument and method verification. No attempt was made to completely characterize these samples, nor to validate origin, quality, or composition.

### GC parameters

GC-FID analyses were performed on a 7890B GC and on an Intuvo 9000 GC. Both systems were equipped with a split/splitless inlet, a flame ionization detector, and an Agilent 7693 automatic liquid sampler (ALS) with a 10  $\mu\text{L}$  syringe.

In the reference method, an Agilent J&W DB-HeavyWAX column (30 m × 0.25 mm, 0.25 μm) was used. This polar column offers higher thermal stability than classical PEG or WAX columns, and is especially suitable for the analysis of fragrance constituents eluting between heptane (RI 700) and triacontane (RI 3,000). Table 1 summarizes the reference method parameters.

Two high-throughput methods with a 1.79 and a 4.27 speed gain factor, respectively, were used on the Intuvo 9000 GC. The method parameters obtained using method translation software (part of Agilent OpenLab 2.2 software used for acquisition and data analysis) are given in Table 2 (parameters that were not modified are not listed).

## Results and discussion

### Method transfer from a 7890 GC to an Intuvo 9000 GC

Figures 1A and 1B show the use of the reference method on the 7890 GC and the Intuvo 9000 GC, respectively. As shown, chromatographic resolution is maintained and the same profiles are obtained on both instruments. For selected constituents, the RIs and peak area % (normalization with abstraction of solvent peak) were determined on each instrument. Table 3 summarizes the results, which demonstrate that RI values are the same on both instruments (delta RI <2 RI units). This is important if data acquired on different instruments are to be compared. It also indicates that the Guard Chip incorporated in the Intuvo 9000 GC does not influence relative solute retention. The relative area % data in Table 3 show that comparable quantitative results are obtained on both instruments.

**Table 1.** GC-FID parameters for the reference method on an Agilent 7890 GC and an Agilent Intuvo 9000 GC.

Parameter	Value
Inlet	Split (1/50) - 250 °C, split liner UI (p/n 5190-2295)
Injection Volume	1 μL
Column	Agilent J&W DB-HeavyWAX, 30 m × 0.25 mm, 0.25 μm (p/n 122-7132 or 122-7132-INT)
Column Flow	1.8 mL/min hydrogen, constant flow
Column Temperature Program	50 °C (1 minute) – 5 °C/min – 250 °C (10 minutes)
Intuvo Guard Chip Temperature	Oven track mode
Intuvo Bus Temperature	250 °C
Intuvo Detector Tail	300 °C
Detector	Flame ionization at 300 °C, 40 mL/min hydrogen, 400 mL/min air

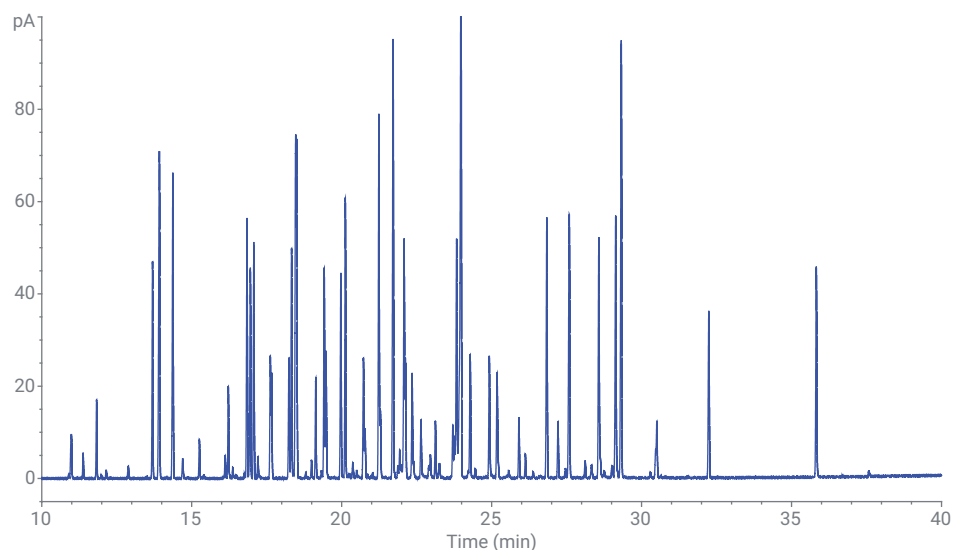
**Table 2.** High-throughput GC-FID parameters for the Agilent Intuvo 9000 GC.

Speed Gain 1.79	
Parameter	Value
Column	Agilent J&W DB-HeavyWAX, 20 m × 0.18 mm, 0.18 μm (p/n 121-7122)
Column Flow	1.3 mL/min hydrogen, constant flow
Column Temperature Program	50 °C (0.56 minutes) – 8.93 °C/min – 250 °C (5.6 minutes)
Speed Gain 4.27	
Parameter	Value
Column	Agilent J&W DB-HeavyWAX, 10 m × 0.18 mm, 0.18 μm (p/n 121-7112)
Column Flow	1.3 mL/min hydrogen, constant flow
Column Temperature Program	50 °C (0.23 minutes) – 21.37 °C/min – 250 °C (2.34 minutes)

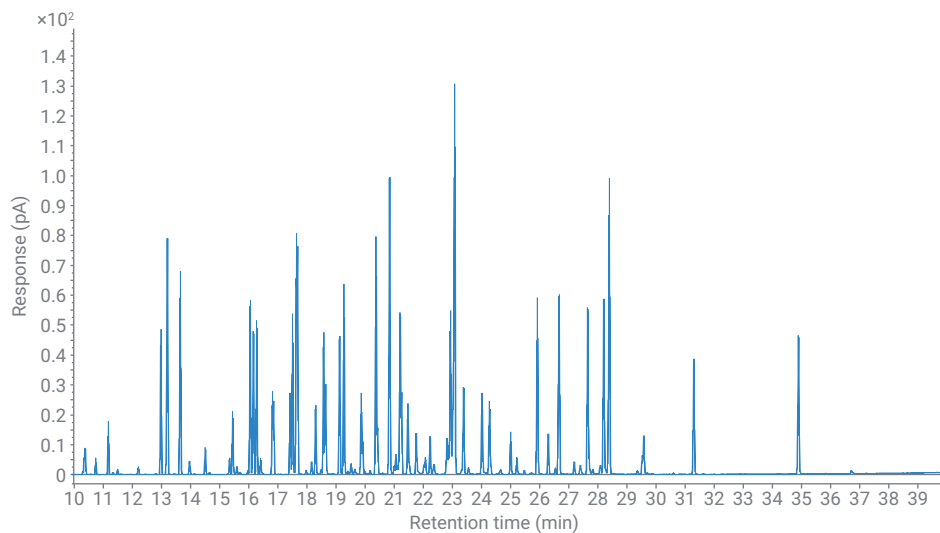
**Table 3.** RI and peak area % for fragrance oil constituents measured with the reference method on an Agilent 7890 GC and an Agilent Intuvo 9000 GC.

Compound	Agilent 7890 GC		Agilent Intuvo 9000 GC	
	RI	Area %	RI	Area %
Linalyl Acetate	1,559	4.02	1,559	3.92
Isobornyl Acetate	1,579	3.12	1,579	3.04
Benzyl Acetate	1,699	2.22	1,699	2.14
Dimethylbenzyl Carbinyl Acetate	1,764	3.47	1,765	3.43
α-Isomethyl Ionone	1,843	2.70	1,841	2.71
Phenylethyl Alcohol	1,898	3.59	1,897	3.55
trans-β-Ionone	1,921	4.70	1,921	4.71
β-Methyl-Ionone	2,039	7.86	2,038	7.75
n-Hexyl salicylate	2,194	2.87	2,193	2.86
Amyl Cinnamaldehyde	2,237	3.38	2,236	3.42
4-Hexenyl-Benzene	2,293	2.76	2,293	2.85
Peonile	2,516	1.93	2,518	1.95
Benzyl Salicylate	2,753	2.32	2,751	2.37

The equivalence of both systems is illustrated by the analysis of a sandalwood oil sample. Sandalwood oil is obtained from steam distillation of wood chips of various species of sandalwood trees (for example, *Santalum album* and *Santalum spicatum*). The oil contains primarily sesquiterpenic alcohols, including  $\alpha$ -santalol and  $\beta$ -santalol. The composition of the oil depends on the species, location, age of tree, season, and extraction process. Relative concentrations of the santalol isomers and other sesquiterpenoids were used for authenticity and QC verification.<sup>6</sup>

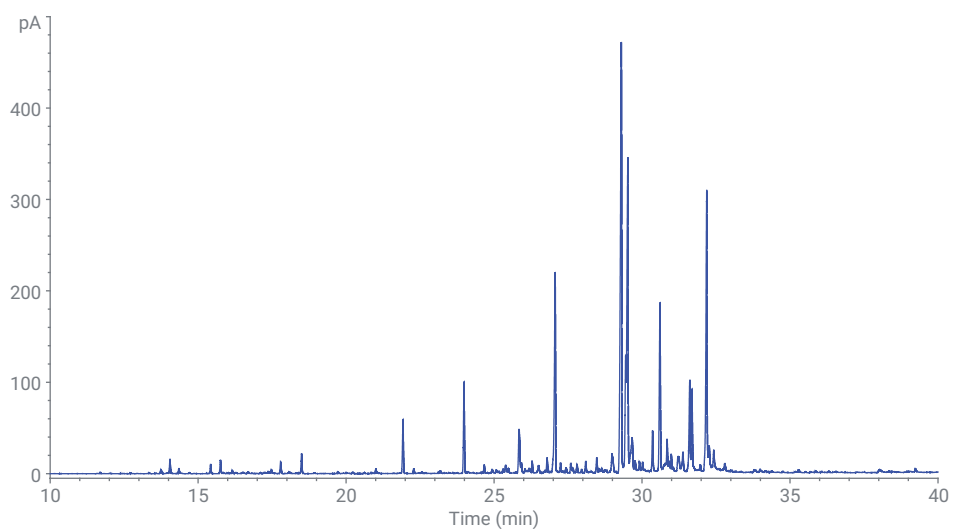


**Figure 1A.** GC-FID analysis of fragrance oil using the reference method on an Agilent 7890 GC with a 30 m  $\times$  0.25 mm, 0.25  $\mu$ m Agilent J&W DB-HeavyWAX column.

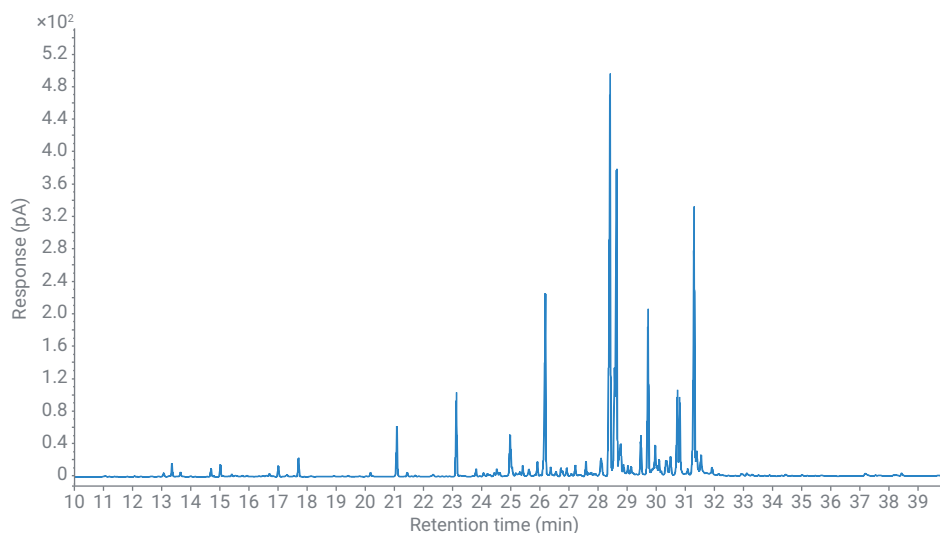


**Figure 1B.** GC-FID analysis of fragrance oil using the reference method on an Agilent Intuvo 9000 GC with a 30 m  $\times$  0.25 mm, 0.25  $\mu$ m Agilent J&W DB-HeavyWAX column.

Figures 2A and 2B show the GC-FID profiles obtained for a commercial sandalwood oil sample on the 7890 GC and Intuvo 9000 GC, respectively. Similar profiles are obtained, and RIs are preserved after method transfer from the 7890 to the Intuvo.



**Figure 2A.** GC-FID analysis of sandalwood oil using the reference method on an Agilent 7890 GC with a 30 m × 0.25 mm, 0.25 μm Agilent J&W DB-HeavyWAX column.



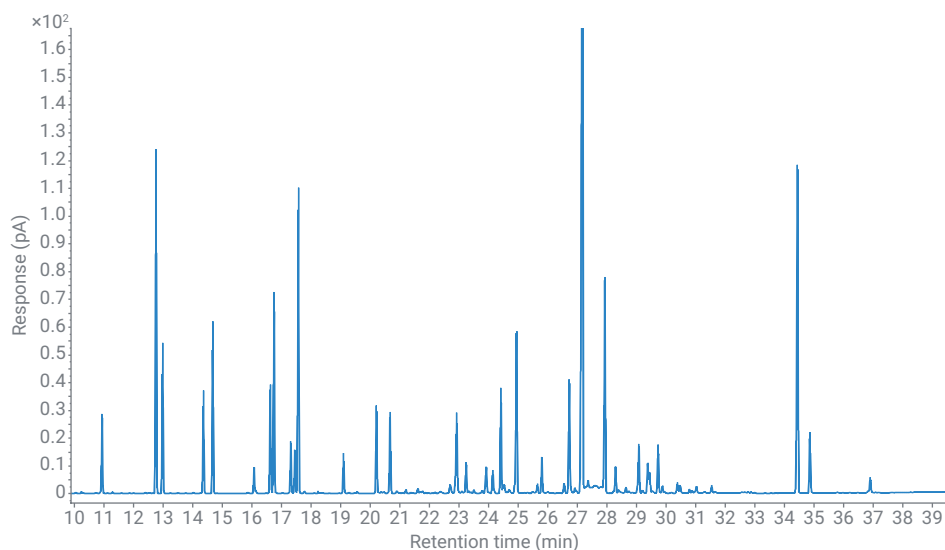
**Figure 2B.** GC-FID analysis of sandalwood oil using the reference method on an Agilent Intuvo 9000 GC with a 30 m × 0.25 mm, 0.25 μm Agilent J&W DB-HeavyWAX column.

### Method translation with maintained resolution

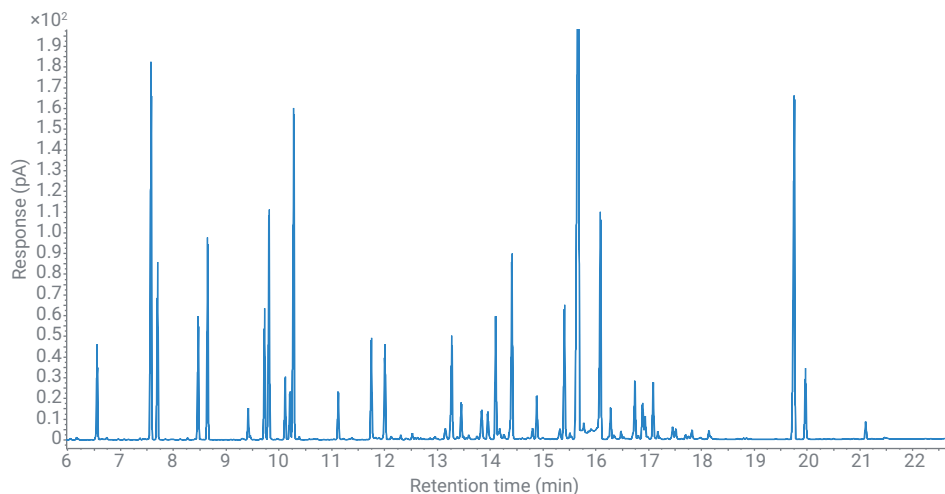
Next, the reference method using a 30 m × 0.25 mm, 0.25 μm DB-HeavyWAX column was translated to a 20 m × 0.18 mm, 0.18 μm DB-HeavyWAX column. Using a shorter column with a smaller id, GC run time can be reduced while chromatographic resolution is maintained.<sup>5</sup> Method parameters for the faster GC method were calculated using the method translation calculator incorporated into the OpenLab GC software. The calculator predicted a 1.79 speed gain factor.

The method translation is illustrated by the analysis of a commercial perfume sample. Figures 3A and 3B respectively show the GC-FID profiles obtained on the 30 m × 0.25 mm, 0.25 μm DB-HeavyWAX column and on the 20 m × 0.18 mm, 0.18 μm DB-HeavyWAX column.

Both profiles are similar, and the chromatographic resolution is maintained. While the last peak elutes at 36.85 minutes using the reference method on the 30 m column, this peak elutes at 21.15 minutes on the 20 m column using the translated method. The measured speed gain factor of 1.74 corresponds well to the predicted speed gain factor (1.79). It was also found that the RIs for the peaks are very similar, within 10 LRI units, which is very acceptable considering that a polar WAX column was used.<sup>4</sup>



**Figure 3A.** GC-FID analysis of perfume using the reference method on an Agilent Intuvo 9000 GC with a 30 m × 0.25 mm, 0.25 μm Agilent J&W DB-HeavyWAX column.

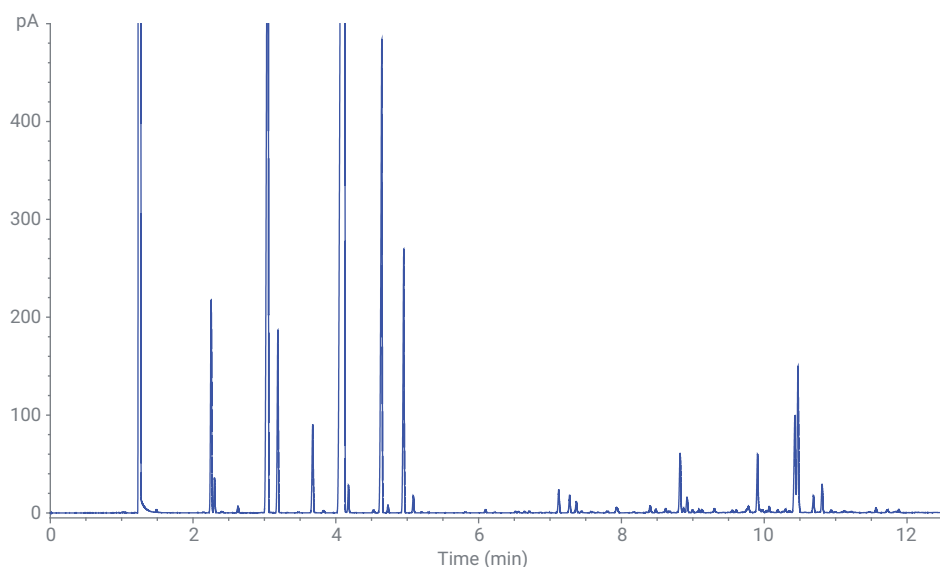


**Figure 3B.** GC-FID analysis of perfume using the translated method on an Agilent Intuvo 9000 GC with a 20 m × 0.18 mm, 0.18 μm Agilent J&W DB-HeavyWAX column.

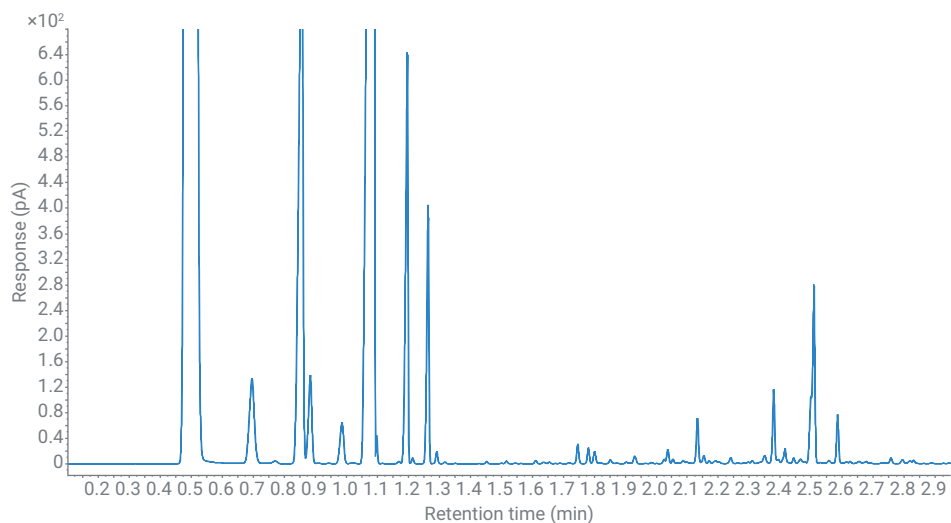
### Method translation to fast GC with reduced resolution

Finally, a high-throughput screening method was used for the analysis of a lemon oil sample. Figure 4A shows the reference profile obtained on a 7890 GC with a 30 m × 0.25 mm, 0.25 μm DB-HeavyWAX column. Figure 4B presents the profile obtained for the same sample on the Intuvo 9000 GC using a 10 m × 0.18 mm, 0.18 μm DB-HeavyWAX column. The method parameters for the high-throughput method were calculated using the method translator, and the predicted speed gain factor was 4.27. The identity of the main peaks, together with the measured RIs and peak area % (normalization after exclusion of solvent peak) are given in Table 4.

The GC-FID profiles shown in Figures 4A and 4B are very similar. The chromatographic resolution was reduced using the short column, as illustrated by the resolution loss for α-pinene/α-thujene (0.69 minutes in the fast GC method, Figure 4B) and for β-bisabolene/geranial (2.50 minutes in the fast GC method, Figure 4B). For most other solutes, including important quality markers such as β-myrcene, *p*-cymene, neral, geranial, and β-bisabolene, sufficient chromatographic resolution was maintained, allowing correct quantification.<sup>7</sup> Geranial eluted at 10.47 minutes using the reference method and at 2.51 minutes using the fast GC method on the Intuvo 9000 GC when using the 10 m column. The measured speed gain factor was 4.17, corresponding well with the predicted speed gain factor. The GC run time was three minutes, resulting in a cycle time below five minutes.



**Figure 4A.** GC-FID analysis of lemon oil using the reference method on an Agilent 7890 GC with a 30 m × 0.25 mm, 0.25 μm Agilent J&W DB-HeavyWAX column.



**Figure 4B.** GC-FID analysis of lemon oil using the translated method on an Agilent Intuvo 9000 GC with a 10 m × 0.18 mm, 0.18 μm Agilent J&W DB-HeavyWAX column.

The selected 180  $\mu\text{m}$  id column also offers sufficient solute capacity, avoiding excessive peak fronting and overloading for the abundant limonene peak (relative concentration of limonene = 60 %). Further reduction of column diameter (for example, to 100  $\mu\text{m}$  id columns) would require reduction of injection volume or increasing the split ratio, with possible loss of detectability of minor solutes, lower accuracy, and lower robustness. Therefore, 150 to 180  $\mu\text{m}$  id columns can be considered the best compromise for resolution, speed of analysis, and quantification.

## Conclusion

High-resolution GC-FID methods for quality control of fragrances, perfumes, and essential oils can be transferred from an Agilent 7890 GC to an Agilent Intuvo 9000 GC with preserved chromatographic profiles and resolution, RIs, and area %. GC run times can be reduced by a factor of two, while chromatographic resolution is maintained, using a shorter narrow bore column if the length-to-id ratio is similar to the reference column. Using shorter GC columns and taking advantage of the fast heating and cooling capabilities of the Intuvo 9000 GC, sample throughput can be increased further, resulting in a fast screening method with a five-minute cycle time.

**Table 4.** RIs and peak area % for lemon oil constituents measured with the reference method on the Agilent 7890 GC, and the fast GC method on the Agilent Intuvo 9000 GC.

Compound	Agilent 7890 GC Reference Method			Agilent Intuvo 9000 GC Fast Method		
	RT (min)	RI	Area %	RT (min)	RI	Area %
$\alpha$ -Pinene	2.25	1021	2.78	0.69	1021	2.82
$\alpha$ -Thujene	2.30	1027	0.65	0.69	1021	–
$\beta$ -Pinene	3.05	1109	16.40	0.85	1107	16.63
Sabinene	3.19	1120	2.09	0.88	1119	2.11
$\beta$ -Myrcene	3.68	1165	1.13	0.98	1167	1.19
Limonene	4.12	1204	59.43	1.08	1200	60.65
$\gamma$ -Terpinene	4.64	1243	6.52	1.19	1241	6.65
<i>p</i> -Cymene	4.95	1267	3.08	1.26	1266	3.16
Neral	9.91	1683	0.83	2.38	1679	0.81
$\beta$ -Bisabolene	10.43	1720	1.34	2.50	1718	1.40
Geranial	10.47	1725	2.00	2.51	1722	2.13

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