

Method Translation for the Analysis of Vanilla Extracts Using an Agilent 8850 GC System with Helium Conservation Module for Carrier Gas Switching

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Abstract

In flavor houses around the globe, analyzing and understanding flavor and fragrance materials' composition is the heart of the work for both quality control (QC) and research and development (R&D) laboratories. This application note highlights the performance of the Agilent 8850 single channel GC for the successful implementation of a common, yet long, GC method for the detailed separation of flavors used in R&D laboratories as well as the successful implementation of a fast method suitable for QC analyses. The use of the Agilent Method Translator software provides users an easy tool to convert chromatographic methods between laboratories such as R&D and QC.¹ The conversion of a relatively long (50-minute) R&D method to a fast (< 5-minute) QC method is demonstrated using both helium and hydrogen as carrier gases. The helium conservation module was used as a gas switching device to allow high sample throughput during the evaluation of the two carrier gases in a single sequence. The gain in efficiency of 10-fold and 14-fold for helium and hydrogen, respectively, did not compromise the chromatographic attributes of the separation or the method's precision and linearity. Lastly, the fast method using hydrogen carrier gas was applied to the analysis of three popular market products: two vanilla extracts and one vanilla flavor. The fast method applied to market products with complex matrices demonstrated the expected performance criteria while showcasing the efficiency of the separation.

Introduction

Flavor and fragrance appeal influences consumers to make decisions every day on the products they purchase. Every year consumers grow more nutrient-conscious, steering away from products formulated with natural and artificially (N&A) manufactured ingredients to embrace ingredients classified as natural or even organic. Vanilla is a flavor variety that has been heavily impacted by changes in consumer habits. It is one of the world's largest selling flavors and can be found in a vast majority of marketable products.² Recent supply and demand pressures on vanilla beans have made it difficult and costly to produce high-quality vanilla extracts for use as standalone products on grocery store shelves or in natural vanilla flavors and fragrances. Under these conditions, it becomes appealing to subtly adulterate vanilla extracts to stretch the natural material's supply; commonly monitored vanilla adulterants include coumarin, ethyl vanillin, eugenol, and guaiacol.^{2,3}

Unfortunately, adulteration impacts the standard of identity for vanilla extracts established by the U.S. Food and Drug Administration. Flavor houses thus rely on QC labs to implement reliable and sensitive analysis techniques for screening incoming raw ingredients to ensure that the materials purchased from ingredient suppliers meet the expected purity standards before the ingredient can enter the flavor formula being manufactured in the near future. Typically, GC-based methods adapted from those used in R&D labs are used, but differences in column length, diameter, and phase can make it challenging to harmonize methods across the R&D and QC functions.

The Agilent Method Translator software is a useful tool for harmonizing GC methods, particularly when each laboratory is using a different GC column, and is included with each GC system and is available for download.¹ The tool allows for direct translation, a translation that considers best efficiency for the two sets of column dimensions being translated between, as well as a speed gain translation option.

This application note demonstrates method translation of a long, R&D-style method to a fast, more efficient QC methodology that suits high sample throughput and simpler quantitation needs. Two sets of method translations are demonstrated, one using helium carrier gas and the other using hydrogen carrier gas. In either case of carrier gas preference, the initial method being translated is the same. Precision and linearity for analytical standards as well as application to vanilla extract varieties showcase the success of the translation.

Experimental

Chemicals and reagents

Five chemicals with vanilla attributes were purchased based on their presence in vanilla extracts, their presence in vanilla flavors, or because they can be detected in vanilla extracts as adulterants. These standards included vanillin ($\geq 97\%$, FCC, FG), ethyl vanillin ($\geq 98\%$, FCC, FG), guaiacol ($\geq 99\%$, FG), eugenol ($\geq 99\%$, FG), and coumarin crystalline, all obtained from Sigma-Aldrich (St. Louis, MO, USA). Also from Sigma-Aldrich, anhydrous decane ($\geq 99\%$) was used as an internal standard and 200-proof anhydrous ethanol ($\geq 99.5\%$) was used as the solvent in the prepared standards and diluent of the vanilla extracts.

Three commonly branded varieties of vanilla extracts—pure vanilla extract, organic vanilla extract, and vanilla flavor (artificially flavored)—were purchased from an online retailer to apply the translated methods to popular market products that required minimal sample preparation.

In-house hydrogen with 99.9999% purity specification was the source for the hydrogen carrier gas methods, while in-house helium with similar specifications was the source of the helium carrier used in the helium methods.

Repeatability studies were conducted with a multi-component standard containing each analyte at 100 ppm, including decane as the internal standard, also at 100 ppm. Later in the work, precision as it pertained to the vanilla extracts was also present where the market products were diluted 10-fold.

Linearity studies were conducted from 10 to 100,000 ppm (10% v/v) in 10-fold increments. The 100,000 ppm standards were prepared individually in ethanol for each of the five analytes. The 10, 100, 1,000, and 10,000 ppm standards were prepared serially in ethanol. Decane was included in each of the standards at an equivalent concentration to the analytes.

For quantitation of the analytes present in the store-bought vanilla extract samples, a working calibration range of 100 to 5,000 ppm was created. Multi-component standards at 500 and 5,000 ppm were prepared from the previously made 1,000 and 10,000 ppm solutions used in the method linearity experiments. Aliquots of the 100 and 1,000 ppm solutions were vialled directly from the method linearity samples previously prepared.

Instrument and methods

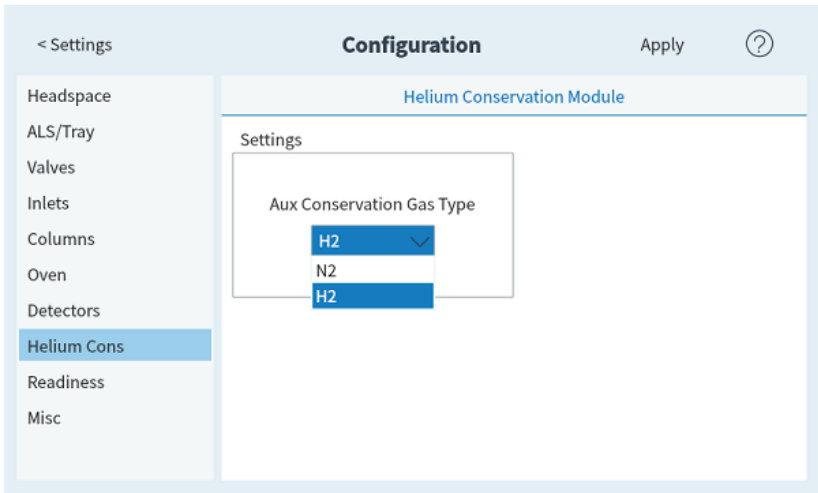
The entirety of this study was performed on an Agilent 8850 GC system equipped with a split/splitless (S/SL) inlet, a helium conservation module for carrier gas switching (Figure 1), and a flame ionization detector (FID). Using the helium conservation module as a carrier gas switching device allows users to plumb two carrier gases to the module's inputs to allow the method to dictate which gas should be supplied to the S/SL inlet as carrier by any given method in a sequence table.



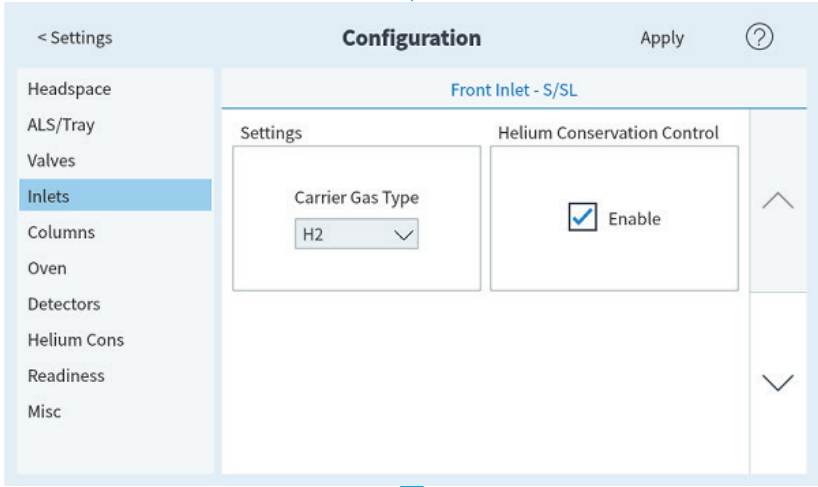
Figure 1. Agilent helium conservation module where the AUX channel supports hydrogen or nitrogen as a second carrier gas to helium.

In-house helium was plumbed to the He channel of the helium conservation module, while in-house hydrogen was plumbed to the AUX channel on the helium conservation module (Figure 2). The Output channel was plumbed directly to the S/SL pneumatic module's carrier gas channel.

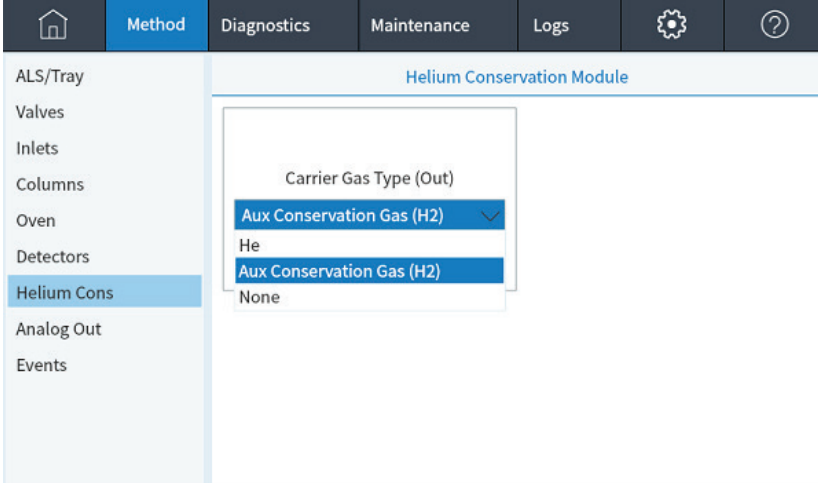
A



B



C



The figure consists of three screenshots (A, B, and C) from the Agilent 8850 local user interface, showing the configuration of the helium conservation module. Screenshot A shows the 'Helium Conservation Module' configuration page with the 'Aux Conservation Gas Type' dropdown menu open, displaying 'H2', 'N2', and 'H2'. Screenshot B shows the 'Front Inlet - S/SL' configuration page with the 'Carrier Gas Type' dropdown menu set to 'H2' and the 'Helium Conservation Control' checkbox checked and labeled 'Enable'. Screenshot C shows the 'Helium Conservation Module' configuration page with the 'Carrier Gas Type (Out)' dropdown menu open, displaying 'Aux Conservation Gas (H2)', 'He', 'Aux Conservation Gas (H2)', and 'None'. Blue arrows indicate the flow from A to B and from B to C.

Figure 2. On the Agilent 8850 local user interface, users can easily configure the use of their helium conservation module by selecting the AUX gas of choice (A), enabling the module to control the inlet (B), then choosing within a method which gas they want to be supplied to the inlet for a specific analysis (C).

The helium conservation module plumbed with two carrier gases simplifies GC use by eliminating the need to manually configure and reconfigure gas connections when an alternate carrier is needed, but also adds the ability to leverage two carrier gases within a single sequence. These advantages promote both flexibility and higher sample throughput, resulting in optimized use of the GC system on nights and weekends. Overall, the helium conservation module offers laboratories increased efficiency in day-to-day operations.

Column selection

A 60 m × 0.25 μm, 320 μm inner diameter (id) Agilent J&W DB-1 was the starting point of the method translation. A 60 m column of DB-1 phase is a common column variety used in flavor and fragrance R&D analyses performed by gas chromatography. Quality control laboratories are more suited to use a 10 m, 100 μm id column for the speed and efficiency of their analyses, so a J&W DB-1 with dimensions of 10 m × 100 μm id, 0.1 μm film was chosen. To demonstrate a stepwise method translation, two additional J&W DB-1 columns were chosen, keeping the phase ratio the same as the 10 m × 100 μm id column. The additional columns were a 20 m × 180 μm id, 0.18 μm and a 30 m × 250 μm id, 0.25 μm. The phase ratio, which was held constant for the 10, 20, and 30 m columns, was 249.25. Table 1 shows the details of the operating conditions using both helium and hydrogen carrier gas for the four DB-1 columns. Figure 3 demonstrates the use of the method translator and defines how the conditions were derived from the original 50-minute method.

Table 1. Conditions for method translation for both helium and hydrogen carrier gases.

Parameter	Value	
GC System	Agilent 8850 GC with 7693A Automatic Liquid Sampler	
ALS	1 μL injection	
	Solvent A = isooctane, 1 prewash, 1 post wash Solvent B = isooctane, 1 prewash, 1 post wash, 1 sample wash, 6 sample pumps S/SL Syringe: 10 μL (p/n G4513-80203)	
Split/Splitless Inlet	325 °C Septum purge: 3 mL/min 1) 25:1 split 2) 25:1 split 3) 50:1 split 4) 200:1 split	
	– Inlet septa, advanced green, nonstick (p/n 5190-3158) – Low pressure drop split liner (p/n 5183-4647) – Column nut for GC capillaries (p/n 5181-8830) – Column connection – 6 mm using graphite ferrule tool (p/n G3440-80217)	
Helium Conservation Module	– Output channel plumbed to S/SL EPC – AUX conservation gas channel plumbed to house hydrogen (H ₂) gas – Helium channel plumbed to house helium (He) gas	
Column	1) Agilent J&W DB-1 60 m × 320 μm, 0.25 μm (5" cage) (p/n 123-1062E) Graphite Ferrules 0.1 to 0.32 mm column (p/n 5080-8853) 2) Agilent J&W DB-1 30 m × 250 μm, 0.25 μm (5" cage) (p/n 123-1032E) Graphite Ferrules 0.05 to 0.25 mm column (p/n 500-2114) 3) Agilent J&W DB-1 20 m × 180 μm, 0.18 μm (5" cage) (p/n 123-1022E) Graphite Ferrules 0.05 to 0.25 mm column (p/n 500-2114) 4) Agilent J&W DB-1 10 m × 100 μm, 0.10 μm (5" cage) (p/n 123-1012E) Graphite Ferrules 0.05 to 0.25 mm column (p/n 500-2114)	
	Constant flow (He): 1) 1 mL/min 2) 1 mL/min (best efficiency translation) 3) 0.72 mL/min 4) 0.4 mL/min	Constant flow (H ₂): 1) 1 mL/min 2) 1.25 mL/min (best efficiency translation) 3) 0.90 mL/min 4) 0.5 mL/min
Oven	He carrier parameters: 1) 40 °C (hold 0 min), 5 °C/min to 280 °C (2 min hold); run time = 50 min 2) 40 °C (hold 0 min), 12.556 °C/min to 280 °C (0.8 min hold); run time = 19.91 min 3) 40 °C (hold 0 min), 21.136 °C/min to 280 °C (0.48 min hold); run time = 11.84 min 4) 40 °C (hold 0 min), 51.36 °C/min to 280 °C (0.2 min hold); run time = 4.87 min H₂ carrier parameters: 1) 40 °C (hold 0 min), 5 °C/min to 280 °C (2 min hold); run time = 50 min 2) 40 °C (hold 0 min), 14.756 °C/min to 280 °C (0.68 min hold); run time = 16.94 min 3) 40 °C (hold 0 min), 27.109 °C/min to 280 °C (0.37 min hold); run time = 9.22 min 4) 40 °C (hold 0 min), 69.974 °C/min to 280 °C (0.14 min hold); run time = 3.57 min * Optional: In place of post-run bakeout, add a second ramp after 280 °C to 325 °C at 60°C/min to perform an in-run column bakeout	
	Oven equilibration = 1 minute Post run bakeout = 325 °C for 2 minutes (* optional for column bakeout)	
FID	300 °C, H ₂ = 30 mL/min, air = 400 mL/min, N ₂ = 25 mL/min Universal 0.011 inch id FID jet (p/n 5200-0176)	
Data Rate	20 Hz	
Injections in Sequence	Ten injections for 3 days of interday precision; helium injections followed by hydrogen injections	
	Four injections per concentration level in linearity and working calibration; helium injections followed by hydrogen injections	
	Six injections per vanilla extract variety; helium injections followed by hydrogen injections	

Interday precision of the translated method on standards of vanilla analytes was performed for each of the four columns. Linearity was demonstrated on the 30 m and 10 m versions of the DB-1 columns to showcase the injector and detector linearity across a working concentration range of the vanilla analytes for the methods from larger to smaller columns with the speed gain in the analysis methods. Application of the translated method for the 10 m columns to actual samples of vanilla extracts and flavors was performed for precision of real-world samples. Quantitation was executed with a working calibration for the five analytes in these vanilla extract products.

Method Translator

Last file imported:

Speed gain
 Translate
 Best Efficiency

Original Method Parameters		Calculated Method Parameters	
Gas: H2		Gas: H2	
Length (m)	60 m	10 m	
Inner Diameter (µm)	320 µm	100 µm	
Film Thickness (µm)	0.25 µm	0.10 µm	
Phase Ratio	319.25	249.25	
Inlet Pressure (gauge)	4.6893 psi	25.49 psi	
Outlet Flow (mL/min)	1 mL/min	0.5 mL/min	
Average Velocity (cm/s)	18.712 cm/sec	55.902 cm/sec	
Outlet Pressure (abs)	14.696 psi	14.696 psi	
Holdup Time	5.3442 min	0.29814 min	
Outlet Velocity (cm/s)	21.766 cm/sec	111.44 cm/sec	

#	Ramp Rate (°C/min)	Final Temp (°C)	Final Time (min)
Init		40	0
1	5	280	2

Total Run Time: 50.00 min

#	Ramp Rate (°C/min)	Final Temp (°C)	Final Time (min)
Init		40	0
1	69.974	280	0.14

Total Run Time: 3.57 min

Pressure Units: PSI

Original Column Capacity: 3.51

Translated Column Capacity: 0.10

The column capacity of the translated method is 3% of the original column capacity. You may need to adjust your injection volume.

Figure 3. Screenshot showing the method translator used to determine method parameters for the translation of a 60 m DB-1 to a 10 m DB-1 under hydrogen carrier conditions using Best Efficiency.

Results and discussion

Method translation

As demonstrated by the chromatograms of the 100 ppm multi-component standard (Figure 4), good chromatographic peak shape as well as baseline resolution was achieved for the five compounds of interest under both carrier gas conditions for all four chromatographic columns used in this work. Resolution values for analytes of interest were > 4 on the 60 m DB-1 and > 3.5 for the 10 m DB-1 (Table 2). The method translation using helium carrier gas resulted in a 10-fold increase in analysis speed, while the methods translated using hydrogen carrier gas gained a 14-fold increase in analysis speed (Figure 3). Laboratories with SOPs using either carrier gas are offered a significant increase in method efficiency while keeping the same elution profile.

Table 2. Resolution following the 60 m to 10 m column method translation for vanilla analytes at 100 ppm using hydrogen carrier gas.

Compound	60 m Retention Time (min)	Average Resolution (H ₂ Carrier) (n = 10)	10 m Retention Time (min)	Average Resolution (H ₂ Carrier) (n = 10)
Decane (ISTD)	16.254	88.995	1.260	16.471
Guaiacol	18.092	25.169	1.401	18.403
Eugenol	25.574	97.835	1.962	69.526
Unknown Impurity	25.858	3.725	n.d.	n.d.
Vanillin	26.165	4.024	2.010	5.818
Coumarin	27.160	12.183	2.088	9.175
Ethyl Vanillin	27.678	6.444	2.122	3.979

* n.d. = Not detected

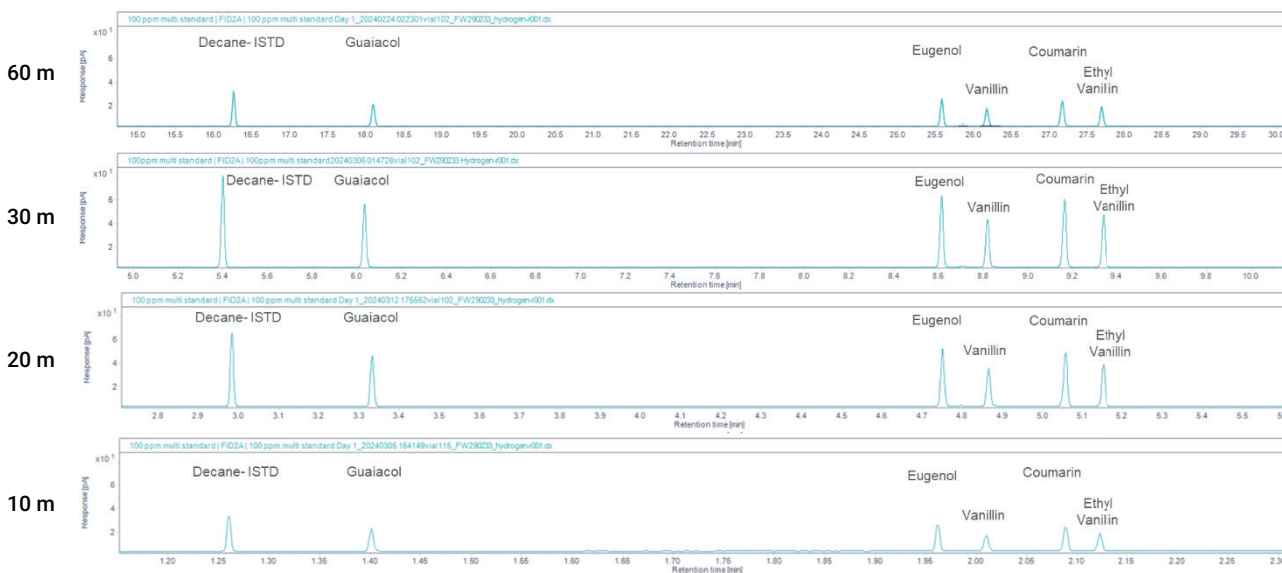


Figure 4. The hydrogen carrier gas method translation from the 60 m to the 10 m DB-1 column using vanilla aroma analytes and internal standard, decane.

Area precision

Area precision of the five analytes of interest was evaluated for each of the four columns used in the method translation over three days with 10 injections per day per carrier gas. Results from the 10 injections per day per carrier were normalized to the internal standard, averaged, then tabulated.

Across all five analytes and all four translated methods studied for the different columns, the area precision under helium carrier gas was < 1.85% RSD, and < 2.5% RSD for hydrogen carrier gas (Table 3). Having low precision for the analytes of interest using both carrier gases gives confidence that the method can reliably handle the separation on each type of column used on a one-time basis as well as over the course of several days of repeated measurements.

Method calibration

Linearity is demonstrated using the 30 m DB-1 and the 10 m DB-1 methods. Each of the five analytes of interest were studied from 10 to 100,000 ppm using both helium and hydrogen carrier gases. A calibration range demonstrating linear behavior across a wide concentration range is critical, because the use rate of the five compounds can be quite large depending on their final application. The concentration of these compounds in an extract versus a flavor can be variable. Their concentration in a flavor going into different food products or fragrances can be even more variable, so having a method suitable across a large

concentration range is highly desirable. The lowest R^2 achieved was 0.9997, while several compounds achieved $R^2 = 1.0000$. Figure 5 shows an example of the linear regression model for vanillin for the 10 m DB-1 method under both

carrier gas conditions. From Table 4, it is evident that linearity was maintained from columns of larger dimensions to ones of much smaller length and diameter, while gaining a significant decrease in analysis times.

Table 3. Interday area precision (%RSD) for the four Agilent J&W DB-1 columns translated using hydrogen carrier gas.

Average Area Precision (%RSD) (n = 10 Injections/Day)					
Column	Guaiacol	Eugenol	Vanillin	Coumarin	Ethyl Vanillin
Day 1					
60 m	2.114	1.789	1.487	1.487	1.597
30 m	0.885	0.956	1.013	0.930	0.997
20 m	0.649	0.801	0.844	0.849	0.850
10 m	1.125	0.818	0.995	0.966	1.067
Day 2					
60 m	1.429	1.537	1.424	1.595	1.594
30 m	0.707	0.676	1.227	0.732	0.970
20 m	0.467	0.560	0.646	0.509	0.621
10 m	1.119	0.920	1.096	0.908	0.984
Day 3					
60 m	1.224	1.357	2.441	1.780	2.223
30 m	0.792	1.110	1.434	1.170	1.317
20 m	0.590	0.655	0.722	0.569	0.666
10 m	0.582	0.740	0.746	0.749	0.900

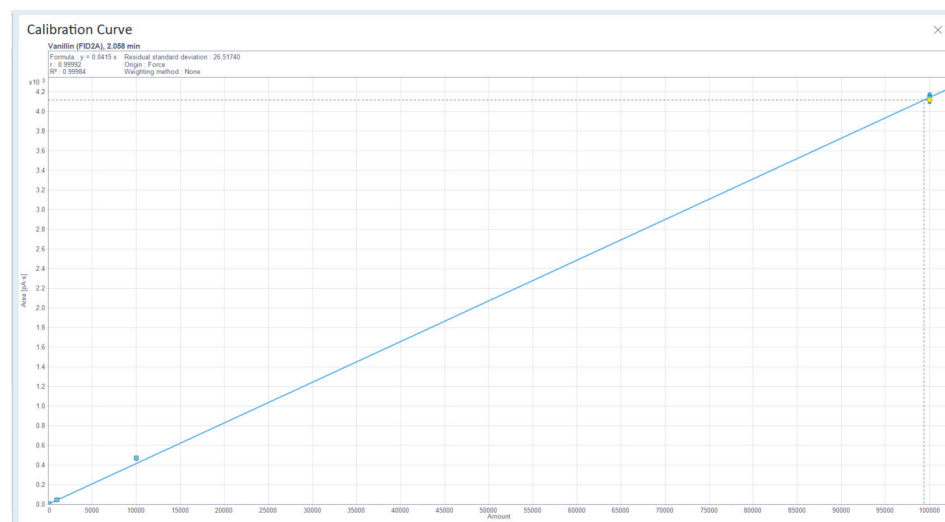


Figure 5. An example of the full range calibration curve, 10 to 100,000 ppm for vanillin, on an Agilent J&W DB-1, 10 m column using hydrogen carrier gas.

Vanilla extracts and flavor

Applying the analytical method for the 10 m DB-1 column to two vanilla extracts and one vanilla flavor bought from an online retailer was an additional demonstration of success of the method translation using both carrier gases. Just as in the method translation, studies of area precision and linear behavior across a working calibration range were important. Quantification of the vanilla aroma compounds of interest detected in the extracts and flavor was also an important final aspect of this application note.

The area precision of six injections of each extract and flavor under both carrier gas conditions was < 4% RSD for the detectable analyte area on the 10 m DB-1 method (Table 5). The area precision demonstrates that the translated method was successful when applied to samples of vanilla extract and vanilla flavor, each of which possess more complex matrices than analytical standards.

To better quantify any detectable quantities of the five vanilla aroma compounds in the extracts and flavor specifically, a working calibration range was studied from 100 to 5,000 ppm in addition to the overall method calibration previously discussed. The tighter concentration range was chosen because it is more relevant to the expected analyte concentrations in extracts. The R^2 for each of the five analytes in the working calibration range were similar to the performance seen in the wider method calibration (Table 6).

Table 4. The coefficient of determination for the linearity of vanilla analytes ranging from 10 ppm to 100,000 ppm on the 30 m and 10 m DB-1.

Compound	R^2	
	Helium Carrier Gas	Hydrogen Carrier Gas
30 m DB-1 (n = 4)		
Guaiacol	0.9999	0.9999
Eugenol	1.0000	0.9999
Vanillin	0.9997	0.9997
Coumarin	0.9998	0.9998
Ethyl Vanillin	1.0000	0.9999
10 m DB-1 (n = 4)		
Guaiacol	1.0000	1.0000
Eugenol	0.9999	0.9999
Vanillin	0.9998	0.9998
Coumarin	0.9998	0.9998
Ethyl Vanillin	1.0000	1.0000

Table 5. Area precision (%RSD) of detectable analytes in three real-world vanilla products.

Column	Guaiacol	Eugenol	Vanillin	Coumarin	Ethyl Vanillin
Helium Carrier Gas, 10 m (n = 6)					
Pure Vanilla Extract	n.d.	n.d.	1.263	n.d.	n.d.
Organic Vanilla Extract	n.d.	n.d.	1.009	n.d.	n.d.
Artificial Vanilla Flavor	n.d.	n.d.	3.812	n.d.	2.631
Hydrogen Carrier Gas, 10 m (n = 6)					
Pure Vanilla Extract	n.d.	n.d.	0.862	n.d.	n.d.
Organic Vanilla Extract	n.d.	n.d.	0.342	n.d.	n.d.
Artificial Vanilla Flavor	n.d.	n.d.	1.269	n.d.	0.902

* n.d. = not detected

Table 6. Coefficients of determination for vanilla aroma analytes ranging from 100 to 5,000 ppm on the 10 m Agilent J&W DB-1 column, n = 4.

Compound	R^2	
	Helium Carrier Gas	Hydrogen Carrier Gas
Guaiacol	0.9998	0.9998
Eugenol	0.9999	1.0000
Vanillin	0.9999	0.9998
Coumarin	0.9999	0.9999
Ethyl Vanillin	0.9998	0.9998

Each of the extracts and artificial flavors required a 10-fold dilution in ethanol to reduce the matrix effects on the consumables in the inlet as well as to reduce the vapor volume of the injection. The extracts can have a significant water content that can cause the vapor volume to expand in the inlet upon injection, possibly resulting in inconsistent mass on column. The nonvolatile components of the extracts' matrix compromise inlet liners when left undiluted, essentially treating the packing of inlet liners like a filter within the sample flow path. This also affects the consistency of mass on column when studying the precision of multiple injections.

While the chromatography of the vanilla extracts and flavor (Figure 7) indicates that many analytes beyond vanillin and ethyl vanillin have the potential to be detected, even the analytes described as possible adulterants, quantitation was not reliable as these signals had a signal-to-noise ratio (S/N) below 10; 10 is the threshold for limit of quantitation (LOQ).

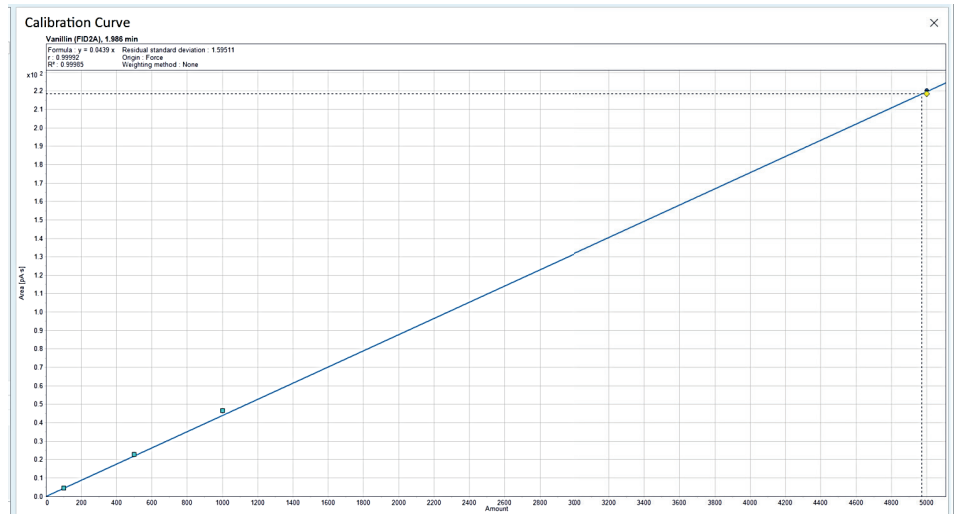


Figure 6. An example of the working calibration range, 100 to 5,000 ppm vanillin, on an Agilent J&W DB-1, 10 m column using hydrogen carrier gases.

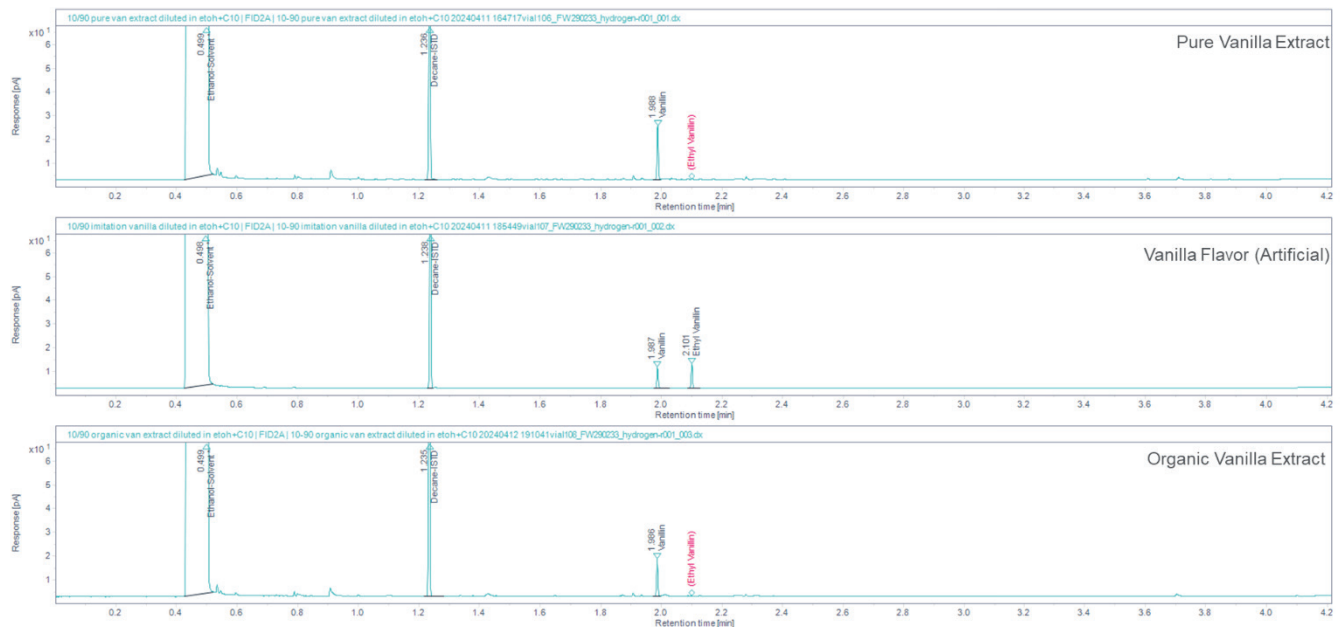


Figure 7. Representative chromatograms of three vanilla extract market products from the translated method for the 10 m Agilent J&W DB-1 column.

For low-level signals that may be present, a mass spectral detector (MSD) would be a preferred secondary source of peak identification; this is because it is possible that low-level signals are analytes separated from the extracts' complex matrices that possess similar retention behavior yet are not an adulterant from the analytes of interest. Each market product presented quantifiable levels of vanillin (Figure 8) such that the analytes had S/N > 10 and the retention times matched those of the standards. It was only the artificial flavor that was formulated with one additional analyte, ethyl vanillin, but this was not unexpected; artificial or imitation products can formulate with natural sources of ethyl vanillin without compromising the standard of identity of the flavor.

Conclusion

This application note describes the successful analysis of a subset of vanilla aroma compounds using the Agilent 8850 GC System for a relatively long R&D method suitable for complex flavor matrices. The analysis of the same vanilla aroma compounds was also demonstrated successfully with a method that was less than five minutes long. The fast method is appreciated by laboratories centered around efficiency and high sample throughput, such as the workflow of QC laboratories in flavor houses. The GC users in QC laboratories are using shorter length, smaller diameter columns because they are mainly analyzing for purity content of incoming raw ingredients before they are added to a flavor formula being manufacturing in the coming hours. It is for this reason that fast methods are critical to the day-to-day operations of flavor manufacturers.

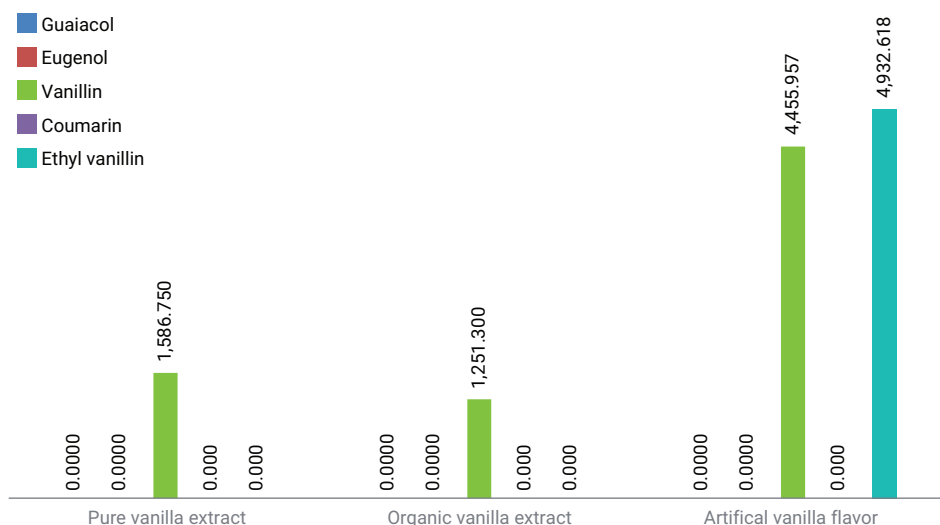


Figure 8. Detectable analyte concentration (ppm) for three vanilla extract market products using hydrogen carrier gas.

To facilitate the conversion of a method that is 50 minutes long to one that is < 5 minutes, the method translator was used for an easy conversion of method parameters as well as pairing the 8850 GC System with a helium conservation module to use as a gas switching device for users that prefer to operate with alternative carrier gases, such as hydrogen. Using this module, users have the flexibility to switch carrier gases line by line in a sequence table as opposed to having to manually change gases plumbed to the inlet or waiting until a sequence is over to switch to an alternative carrier gas. The flexibility and time savings the helium conservation module offers laboratories results in increased analytical efficiency and opportunity for higher sample throughput.

The resulting speed gain in analysis from the method translation of the long method to the fast method was 10-fold for helium carrier gas methods and 14-fold for hydrogen carrier gas methods. Whether we discuss the long method or the fast method, helium carrier gas or hydrogen carrier gas, the performance of the 8850 GC as demonstrated by the five vanilla aroma compounds produced baseline resolution values greater than 3.5, interday area precision below 2.5% RSD, and linearity with $R^2 \geq 0.9997$, with several compounds achieving $R^2 = 1.0000$.

References

1. Agilent GC calculator and method translator software available for download from: <https://www.agilent.com/en/support/gas-chromatography/gccalculators>
2. Cristina, M. M.-L.; *et al.* Prediction of coumarin and Ethyl Vanillin in Pure Vanilla Extracts Using MID-FTIR Spectroscopy and Chemometrics. *Talanta* **2019**, *197*, 264–269. DOI: [10.1016/j.talanta.2019.01.033](https://doi.org/10.1016/j.talanta.2019.01.033).
3. Lingxia, X.; *et al.* Advances in the Vanillin Synthesis and Biotransformation: A Review. *Renewable and Sustainable Energy Reviews* **2024**, *189(A)*. DOI: [10.1016/j.rser.2023.113905](https://doi.org/10.1016/j.rser.2023.113905)

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Printed in the USA, August 5, 2024
5994-7401EN