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# Method Translation for the Analysis of Vanilla Extracts Using a Compact, Single Channel GC-FID and Carrier Gas Switching Module

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## Introduction

Every year consumers grow more nutrient-conscious, steering their consumption habits towards ingredients labeled as natural or even organic. This trend even extends into flavors that are called out in an ingredient list on a food or beverage product.

Vanilla is a flavor variety that has been heavily impacted by changes in consumer habits as well as by supply and demand of vanilla beans used to produce high-quality vanilla extracts. Unfortunately, these market conditions, increase the likelihood of adulteration in vanilla extracts by use of aroma chemicals that are not naturally occurring. Common adulterants are coumarin (C), ethyl vanillin (EV), eugenol (E), and guaiacol (G).[2,3] Adulteration impacts the standard of identity for vanilla extracts established by the U.S. FDA; therefore, flavor companies rely on QC labs to implement reliable and sensitive analysis techniques for screening incoming raw ingredients against expected purity standards before the ingredient can enter a flavor formula on the manufacturing floor. Screening is typically executed with gas chromatography (GC)-based methods adapted from those used in a R&D lab. However, differences in column length, diameter, and phase can make it seem challenging to harmonize GC methods across the R&D and QC functions within a company due to the different analysis objectives of the respective laboratories.

### Experimental Goals-

- Demonstrate the ease of the Agilent Method Translator in harmonizing GC methods for multiple columns and gases
- Establish method precision, linearity, and resolution on standards of vanilla-like adulterants using the translated methods
- Successfully showcase the method translation using store-bought extracts to verify the integrity of the fastest GC method on samples containing matrix
- Screen for and quantify any vanilla adulterants in the samples of vanilla extracts with the goal of detecting no adulterants using the fastest GC method.

## Experimental

Table 1. GC Parameters used in the Method Translator

| Parameter | Value |
|---|---|
| GC System | Agilent 8850 GC with 7693A Automatic Liquid Sampler |
| ALS | 1 µL injection |
| | Solvent A = isooctane, 1 prewash, 1 post wash<br>Solvent B = isooctane, 1 prewash, 1 post wash, 1 sample wash, 6 sample pumps<br>S/SL Syringe: 10 µL (p/n G4513-80203) |
| Split/Splitless Inlet | 325 °C<br>Septum purge: 3 mL/min<br>1) 25:1 split<br>2) 25:1 split<br>3) 50:1 split<br>4) 200:1 split |
| | – Inlet septa, advanced green, nonstick (p/n 5190-3158)<br>– Low pressure drop split liner (p/n 5183-4647)<br>– Column nut for GC capillaries (p/n 5181-8830)<br>– Column connection – 6 mm using graphite ferrule tool (p/n G3440-80217) |
| Helium Conservation Module | – Output channel plumbed to S/SL EPC<br>– AUX conservation gas channel plumbed to house hydrogen ($H_2$) gas<br>– 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)<br>Graphite Ferrules 0.1 to 0.32 mm column (p/n 5080-8853)<br>2) Agilent J&W DB-1 30 m × 250 µm, 0.25 µm (5" cage) (p/n 123-1032E)<br>Graphite Ferrules 0.05 to 0.25 mm column (p/n 500-2114)<br>3) Agilent J&W DB-1 20 m × 180 µm, 0.18 µm (5" cage) (p/n 123-1022E)<br>Graphite Ferrules 0.05 to 0.25 mm column (p/n 500-2114)<br>4) Agilent J&W DB-1 10 m × 100 µm, 0.10 µm (5" cage) (p/n 123-1012E)<br>Graphite Ferrules 0.05 to 0.25 mm column (p/n 500-2114) |
| | **Constant flow (He):**<br>1) 1 mL/min<br>2) 1 mL/min (best efficiency translation)<br>3) 0.72 mL/min<br>4) 0.4 mL/min<br>**Constant flow ($H_2$):**<br>1) 1 mL/min<br>2) 1.25 mL/min (best efficiency translation)<br>3) 0.90 mL/min<br>4) 0.5 mL/min |
| Oven | **He carrier parameters:**<br>1) 40 °C (hold 0 min), 5 °C/min to 280 °C (2 min hold); run time = 50 min<br>2) 40 °C (hold 0 min), 12.556 °C/min to 280 °C (0.8 min hold); run time = 19.91 min<br>3) 40 °C (hold 0 min), 21.136 °C/min to 280 °C (0.48 min hold); run time = 11.84 min<br>4) 40 °C (hold 0 min), 51.36 °C/min to 280 °C (0.2 min hold); run time = 4.87 min<br>**$H_2$ carrier parameters:**<br>1) 40 °C (hold 0 min), 5 °C/min to 280 °C (2 min hold); run time = 50 min<br>2) 40 °C (hold 0 min), 14.756 °C/min to 280 °C (0.68 min hold); run time = 16.94 min<br>3) 40 °C (hold 0 min), 27.109 °C/min to 280 °C (0.37 min hold); run time = 9.22 min<br>4) 40 °C (hold 0 min), 69.974 °C/min to 280 °C (0.14 min hold); run time = 3.57 min<br>* 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<br>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<br>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 |

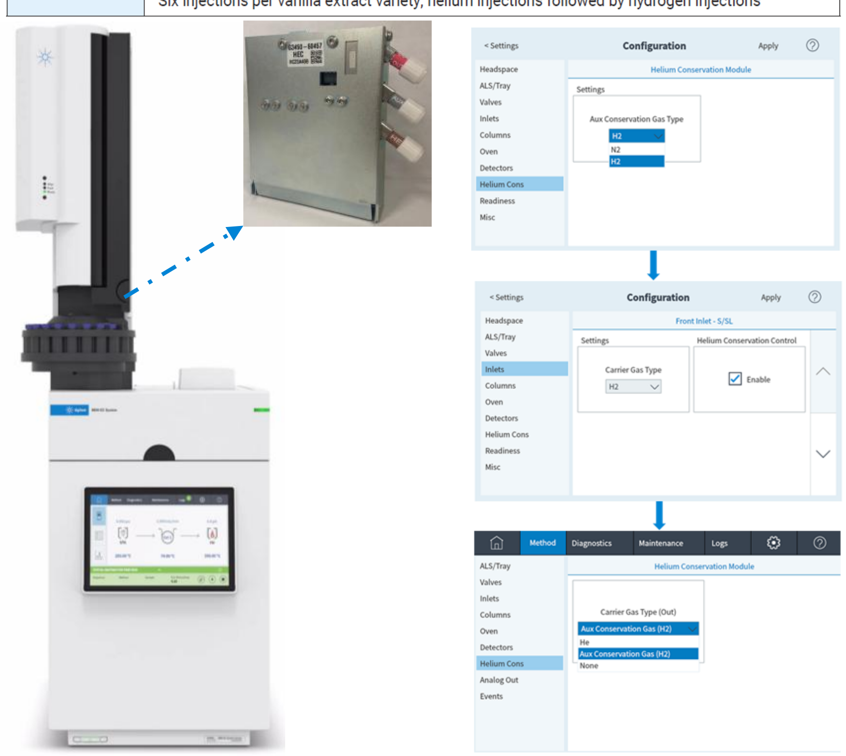


Figure 1. Agilent 8850 GC equipped with a helium conservation module where the AUX channel is configured to use $H_2$ as a second carrier gas for sequence efficiency

## Results and Discussion

### Method Translation-

A multi-standard of vanillin, an expected analyte, along with the four possible vanilla adulterants was prepared at 100 ppm. The method translation achieved good chromatographic peak shape and baseline resolution (Figure. 3 and Table 2) using both He and $H_2$ carrier gases across four DB-1 columns of varying lengths.

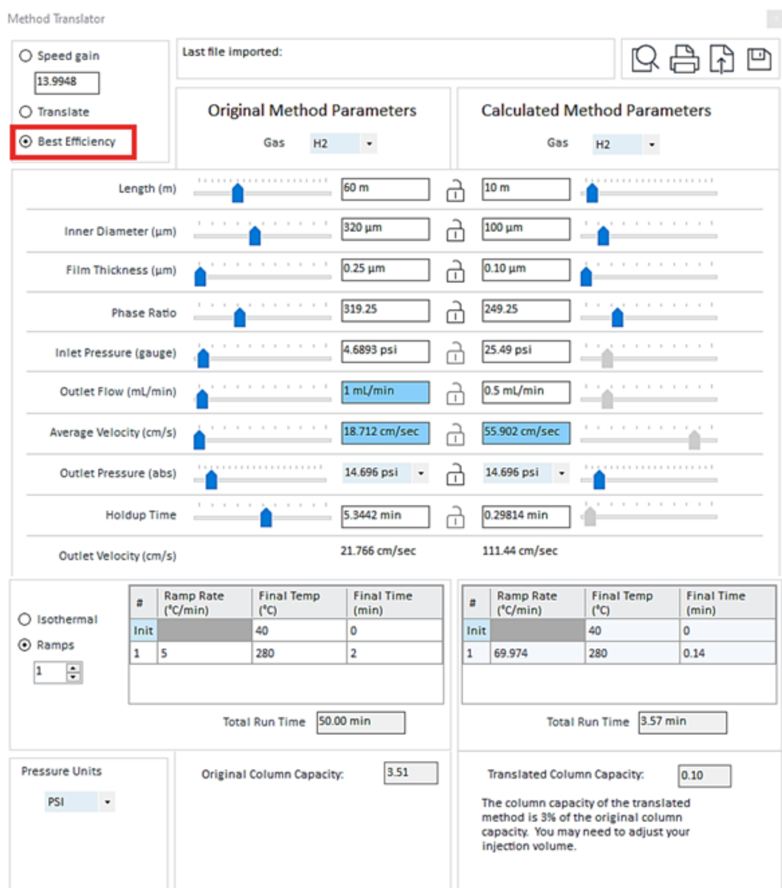


Figure 2. Agilent Method Translator for the translation of a 60 m to a 10 m DB-1

Table 2. Resolution of analytes at 100 ppm during the 60 m to 10 m method translation

| 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

### Interday Area Precision-

Area precision was conducted on the analytes with ten injections per day per carrier gas then were normalized to the internal standard and averaged over three days of experimentation (Table 3).

Table 3. Interday precision (%RSD) for the four J&W DB-1 columns using $H_2$ 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 |

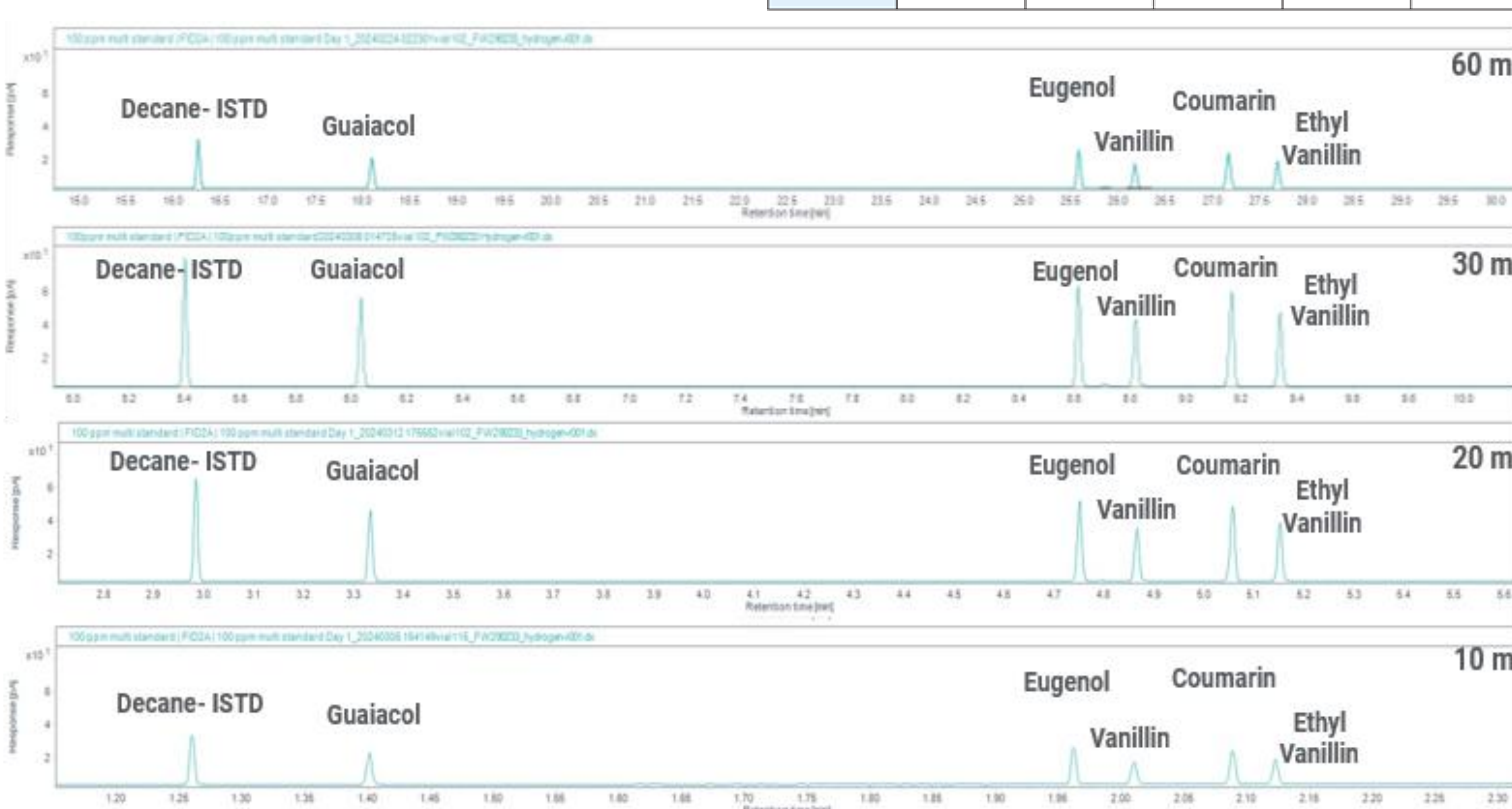


Figure 3. Method translation with $H_2$ carrier gas, four columns and five vanilla-like analytes

## Results and Discussion

### Method Linearity-

Linearity is demonstrated for each of the analytes of interest from 10 to 100,000 ppm using both He and $H_2$ carrier gases. Demonstrating linear behavior across a wide concentration range is critical because the use rate of the adulterants can be quite large and variable; therefore, a method suitable across a large concentration range is highly critical in the flavor space. The $R^2$ ranged from 0.9997 to 1.000

### Analysis of Vanilla Extracts and Flavor-

The area precision of each extract and flavor under both carrier gas conditions on a 10 m DB-1 was < 4% RSD for the detectable analyte area. It is important to assess precision of the fast analytical methods with the vanilla extracts to look for potential matrix effects that would make the translated method harder to use in the QC environment of a flavor house.

### Quantification of Detectable Analytes-

To better quantify any detectable quantities of the five vanilla aroma compounds in store bought samples, a working calibration range in addition to method linearity was studied from 100 to 5,000 ppm. The tighter concentration range was chosen because it is more relevant to the expected analyte concentrations in these store-bought products. Coefficients of determination ranged from 0.9998 to 1.000.

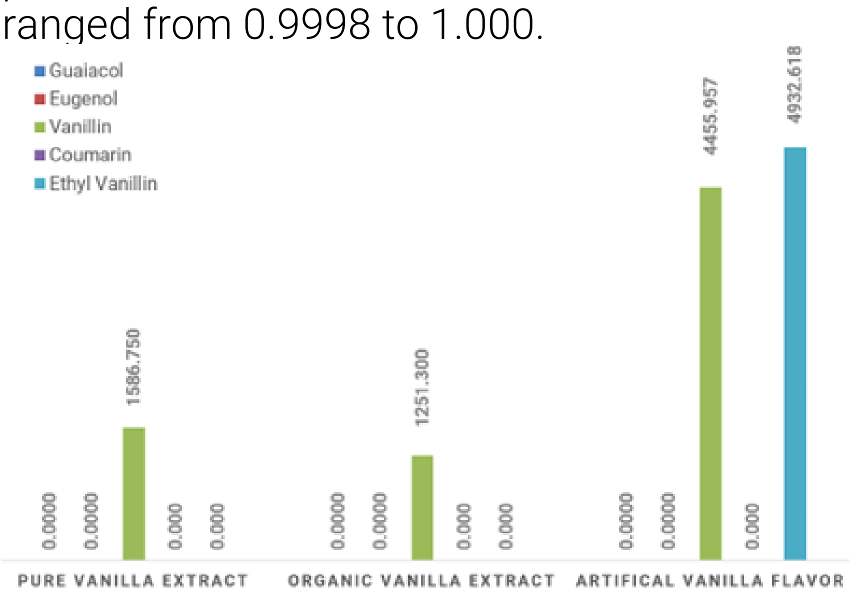


Figure 5. Detectable analyte concentration (ppm) for three vanilla extract market products using H2 carrier gas

| Compound | $R^2$ Helium Carrier Gas | $R^2$ Hydrogen Carrier Gas |
|---|---|---|
| 30-meter 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-meter 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 4. Coefficient of determination for linearity ranging 10 to 100,000 ppm on the 30 m and 10 m DB-1

## Conclusions

### Method Translation-

- The method translator tool provided an easy conversion of a 50-minute-long method to one that is < 5 minutes resulting in a speed gain of 10-fold and 14-fold, for He and $H_2$ carrier gases, respectively (Figures 2 and 3)
- Resolution of analytes were >4 on the 60 m and >3.5 on the 10 m DB-1 columns (Table 2)
- Precision was <1.85% RSD and <2.5% RSD for He and $H_2$ carrier, respectively (Table 3)
- Linearity was demonstrated from 10 ppm to 100,000 ppm for He and $H_2$ carrier with $R^2$ ranging from 0.9997 to 1.0000

### Carrier Gas Switching-

- He and $H_2$ carrier gases were alternated line by line in a sequence table for increased analytical efficiency and higher throughput while demonstrating the flexibility the helium conservation module provides its users while also eliminating manual changes to plumbed gases at the GC EPC Module.

## References


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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.
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