An Industrial Push for Hydrogen Carrier Gas in Gas Chromatography: A FAME Application of AOAC Method 996.06

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1. Introduction

Carrier Gases

The limited supply and privatization of helium sales has caused the price to increase exponentially (Figure 1). Suppliers have been forced to severely limit the sale of helium and they are starting to sell exclusively to parties that have no alternative to using helium. Gas chromatography has other options for carrier gas so there are several companies that cannot purchase helium as the suppliers do not view their need to be imperative.

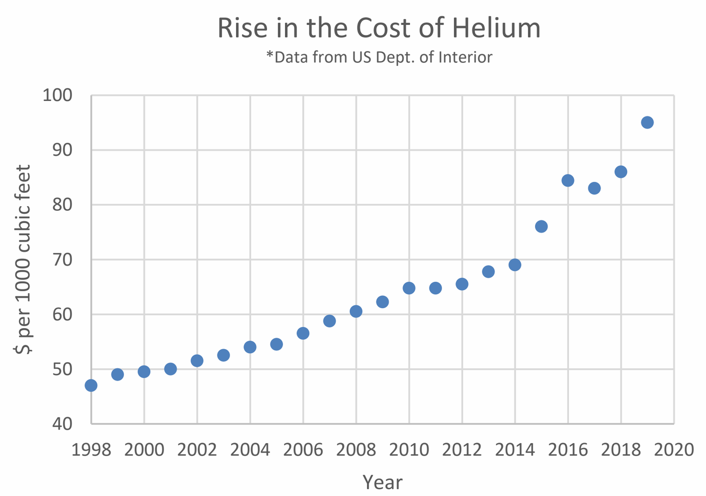

Figure 1: Average helium cost has increased exponentially since 1998.

Industry has had to adapt to the helium shortage by seeking an alternative carrier gas for use with their GCs. The difference in physical properties of the common carrier gas options (i.e. density, viscosity, etc.) makes it so that the use of the same method conditions would have a varying implication on the quality of the chromatography produced. The Van Deemter plot for carrier gases (Figure 2) shows that despite the fact that nitrogen provides the most efficient separation (low HETP = best efficiency), it is at a low linear velocity leading to a longer analysis time. Deviation from the optimal linear velocity with nitrogen has a major implication on the efficiency given that curve is steep. Hydrogen carrier gas has a high optimal linear velocity leading to a faster analysis time. Deviation from the optimal linear velocity has minimal impact on the efficiency of the separation.

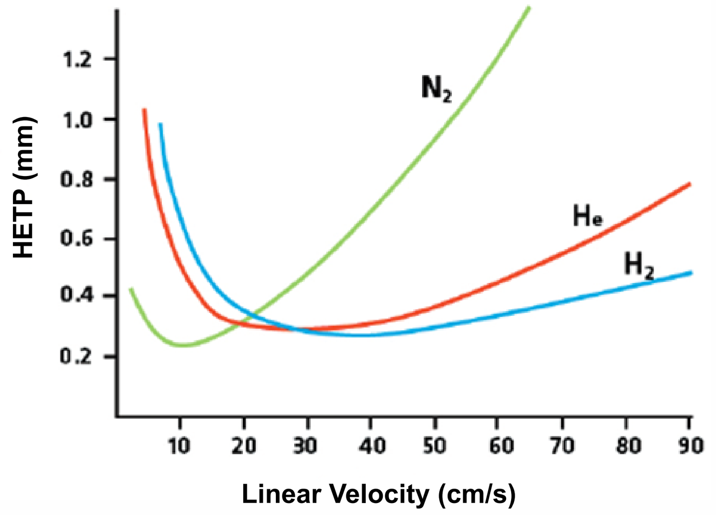

Figure 2: Van Deemter Plot for Carrier Gases
* Column ID dependent

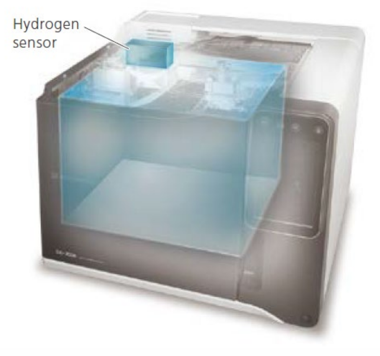

Figure 3: Hydrogen sensor on GC

A common concern regarding hydrogen carrier gas Safety concerns about using hydrogen gas lies with its combustibility. These concerns can be mitigated by the use of a hydrogen sensor as seen in Figure 3. If the hydrogen concentration raises above the 4% (v/v) flash point of hydrogen, the gases will be automatically turned off. This will prevent an emergency even when there are no analysts present to turn off the gas.

Fatty Acids

The United States Food and Drug Administration (FDA) requires the manufacturers of food products to include a profile of fats on the nutrition label (Figure 4). *Trans* and saturated fats are of particular interest given that they have a higher melting point and have the potential to clog arteries if they are consumed in large amounts. Figure 5 shows examples of types of fats.

Saturated Fatty Acid
C14:0

Trans Fatty Acid
C18:2 (t9, t12)

Cis Fatty Acid
C14:1 (c9)

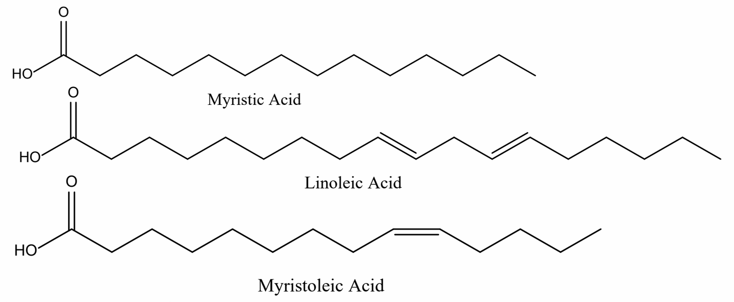

Figure 5: Various fatty acid examples

Nutrition Facts
8 servings per container
Serving size 2/3 cup (55g)
Amount per serving
Calories 230

	% Daily Value*
Total Fat 8g	10%
Saturated Fat 1g	5%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 160mg	7%
Total Carbohydrate 37g	13%
Dietary Fiber 4g	14%
Total Sugars 12g	
Includes 10g Added Sugars	20%
Protein 3g	
Vitamin D 2mcg	10%
Calcium 260mg	20%
Iron 8mg	45%
Potassium 235mg	6%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Figure 4: FDA approved nutrition label

2. Experimental

Fatty Acid Methyl Esters (FAMEs)

Fatty acids contain a carboxylic acid. Their very polar nature and acidity make them incompatible with many column stationary phases. Thus, in order to determine the fat content in food by GC, the AOAC 996.06 method contains a sample preparation procedure that converts the fatty acids to **F**atty **A**cid **M**ethyl **E**sters (FAMEs). The equation in figure 6 shows this conversion.

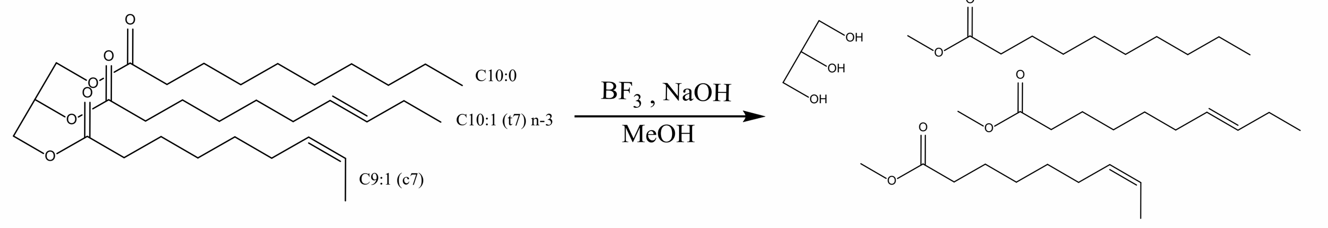

Figure 6: FAME transesterification example as according to AOAC method 996.06

For this work, a Restek 37 component FAME standard (cat # 35077) was purchased and stored in accordance to manufacturer specifications. A serial dilution of the standard allowed for a 5 point calibration curve where the calibration standards that were used were the original concentration as well as 0.50, 0.25, 0.10 and 0.05 times the original concentration.

Instrumentation

For the application, the Shimadzu Nexis GC-2030 unit shown in figure 7 was equipped with Flame Ionization Detector (FID).

The FID components have been optimized in this model to provide better signal response. An improved flow controller yields a more stable baseline such that the sensitivity of the GC-2030 is Shimadzu's flagship GC model.

Figure 7: Shimadzu Nexis GC-2030

GC Method Conditions

The purpose of this work was to compare the chromatography that was yielded from the AOAC method 996.06 which requires the use of helium carrier gas with the results from switching the carrier gas to hydrogen. It was of interest to identify areas of the AOAC method that could be improved upon and do method development such that the result would provide positive peak resolution while reducing the run time. The method conditions for the original method vs the hydrogen method can be seen in table 1.

Table 1: AOAC helium method compared to improved hydrogen method parameters

	Original AOAC Method 996.06	New Nexis GC-2030 Hydrogen Method
Inlet	Split/Splitless; 225 °C; Split Ratio 200:1	Split/Splitless; 225 °C; Split Ratio 200:1
Column	Rt-2560 100 m × 0.25 mmID × 0.20 µm film	Rt-2560 100 m × 0.25 mmID × 0.20 µm film
Carrier	Helium; Constant Linear Velocity 18 cm/s	Hydrogen; Constant Linear Velocity 35 cm/s
Oven	100 °C (4 min hold); 3 °C/min to 240 °C (15 min hold)	150 °C (2 min hold); 4 °C/min to 220 °C; 2 °C/min to 240 °C (8 min hold)
FID	285 °C; H_2 32 mL/min; Air 200 mL/min; Make-up (N_2) 24 mL/min	285 °C; H_2 32 mL/min; Air 200 mL/min; Make-up (N_2) 24 mL/min

3. Results and Discussion

Original AOAC Method 996.06 | *New Nexis GC-2030 Hydrogen Method*

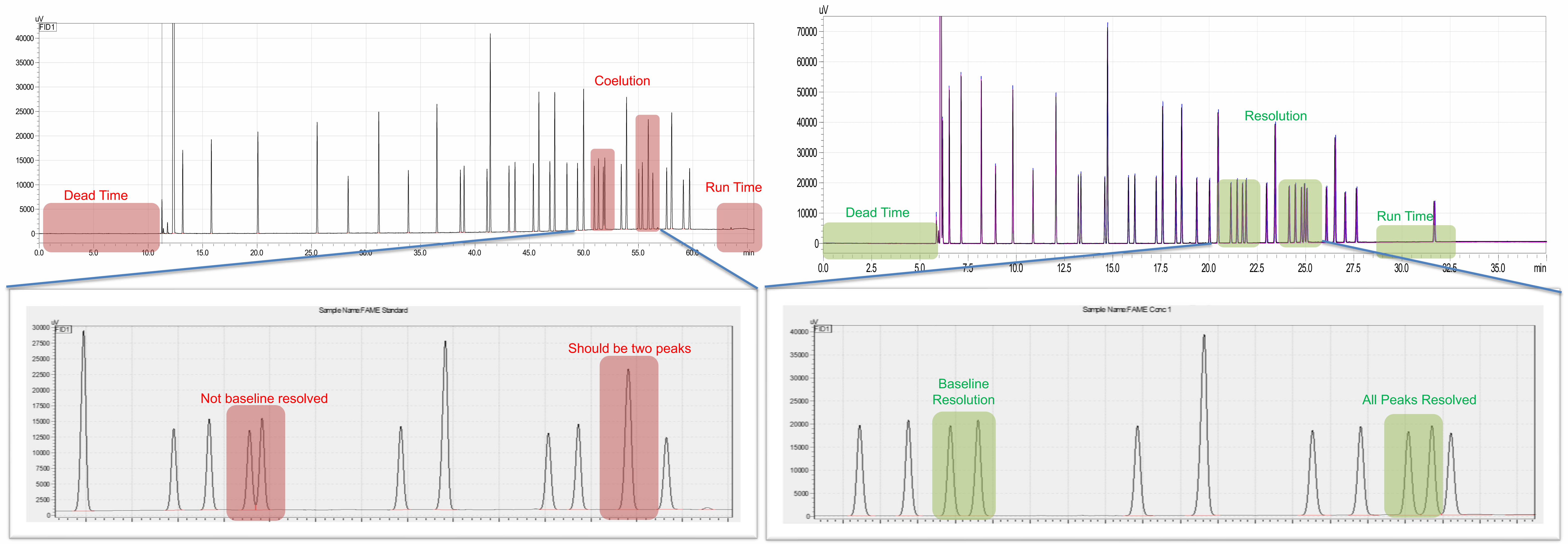

Figure 8: Chromatograms and expanded sections showing the difference between the original helium method and the optimized hydrogen method

In figure 8, the analysis of the same FAMEs standard can be seen run on both helium and hydrogen. What can be seen in using hydrogen is the analysis time can be shortened and the resolution of some of the peaks is increased. Companies that analyze food products for FAME content identify that the more than one hour analysis time makes it difficult to meet production demands and stymies production and profit potential. Taking advantage of the high optimum linear velocity of hydrogen coupled with the extended dead time at the beginning of the run suggests that method development could increase sample turnover. Significant resolution from the early eluting analytes also suggested that the analysis time could be decreased by starting the method at a higher oven temperature with an increased oven ramp.

Sensitivity

The limit of detection and/or the limit of quantitation (LOD/LOQ) is a metric that is typically used to gauge the sensitivity of an analysis. These values are directly correlated to the signal-to-noise ratio where a decrease in signal and/or an increase in noise would lead to poor sensitivity. Given that the carrier gas is the same as the FID fuel gas in the hydrogen method, it follows that the baseline noise is increased in the hydrogen method whereas this phenomenon is not present when using helium. Thus, it is expected that analysts using hydrogen carrier gas can expect a decrease in sensitivity (Figure 9). It is important to note that the reproducibility of hydrogen is of good quality in all standards of concentration above the LOQ (Figure 10).

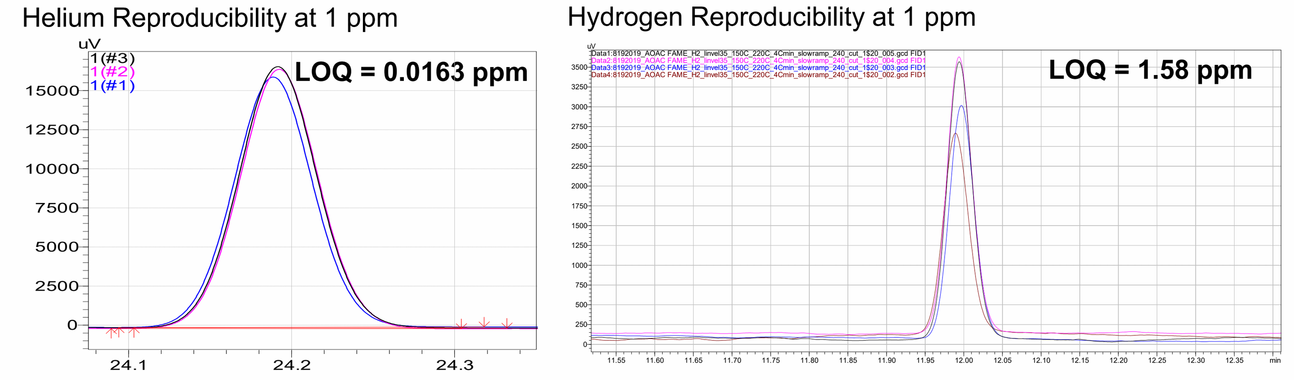

Figure 9: LOQ of the same compound using both helium and hydrogen

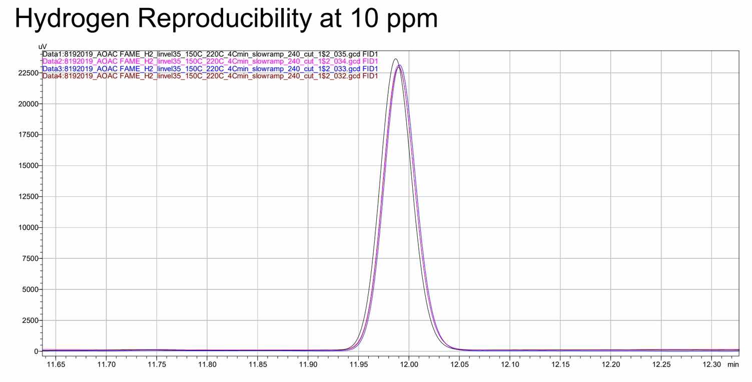

Figure 10: Representation of the same compound above LOQ using hydrogen carrier

4. Conclusions

- It can be financially beneficial to switch to hydrogen carrier gas whether using tank or generator.
- Given that hydrogen has the fastest optimal linear velocity, method development could lead to increased sample throughput which would translate to additional revenue.
- Starting the method at a higher temperature will decrease the GC cycle time which would also lead to a higher profit margin given that the total analysis time would be shortened.
- Although a cheaper alternative to helium, hydrogen carrier does not provide the same sensitivity as helium. This is likely due to the increased baseline noise as the carrier gas used is also the FID fuel gas. In most cases, the loss of sensitivity may not be critical to all labs depending on what level they are trying to reach.