APPLICATION NOTE

No. 21557

Separation of 37 Fatty Acid Methyl Esters Utilizing a High-Efficiency 10 m Capillary GC Column with Optimization in Three Carrier Gases

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Key Words
TR-FAME, fatty acid methyl esters, FAMEs, GC, GC-MS, carrier gas

Goal

To demonstrate the separation of 37 fatty acid methyl esters (FAMEs) on the highly efficient 10 m Thermo Scientific™ TRACE™ TR-FAME GC column, and to show increased sample throughput of up to 400% relative to a 100 m column by optimizing the separation for efficiency and speed using three commonly available carrier gases: nitrogen, hydrogen, and helium.

Introduction

Fats are a major constituent of many foodstuffs including edible oils, meat, fish, grain, and dairy products. They consist of triacylglycerides, which are species that contain glycerol sub-units esterified with aliphatic fatty acid groups (Figure 1).

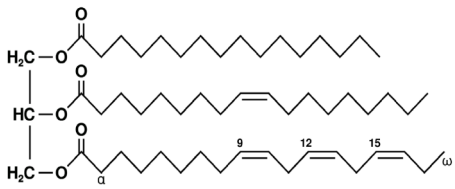

Figure 1. A general triacylglyceride.

The aliphatic chain can vary in carbon length, degree of unsaturation, and isomerization around double bonds giving *cis* and *trans* forms of the fatty acids. *Trans* and hydrogenated fats are important food components that are regularly measured.

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Gas chromatography (GC) is a common method for determining identity and concentration of fatty acids. In order for the fatty acids to be analyzed by GC, the fats in any given matrix require a three-step preparation that includes:

- Extraction from the matrix with a non-polar solvent for clean-up
- Saponification, rendering the free fatty acids
- Derivatization to FAMEs for more amenable analysis

Derivatization of the saponified fatty acids via methylation leads to the formation of the corresponding fatty acid methyl esters (FAMEs), which are the preferred derivatives due to their volatility and high thermal stability. However, separation of the 37 common FAMEs can be difficult to achieve as many differ only slightly in their physical and chemical properties.

Generally, high polarity cyanopropyl or biscyanopropyl chemistries are employed for GC separation to provide the necessary selectivity and resolve all components. In these instances, 100 m columns are often used to provide the required resolution; however, they are expensive, analysis times are extended, and sample throughput is low. This can result in a very high cost of analysis per sample.

TRACE TR-FAME columns have a high polarity phase optimized for FAME analysis. The 70% cyanopropyl polysilphenylene-siloxane phase utilized has a higher operating temperature compared to some other columns and gives extremely low bleed, making it amenable to detection by mass spectrometry.

Here, the advantages of utilizing shorter, high-efficiency FAME columns for this complex analysis are investigated. Higher throughput and potential cost savings for the customer can be realized if the shorter columns provide similar performance and reduced analysis time when compared to commonly used 100 m columns. Additionally, the effects of different carrier gases on the chromatography were investigated to tune the separation for speed or efficiency.

Carrier gas choice has a significant effect on the chromatography. Helium is the most common carrier gas for GC as it is widely available within laboratories, inert, and amenable to MS detection. However, there are instances where hydrogen or nitrogen can be successfully employed to improve a separation.

The modified Golay plot (Figure 2) shows this graphically. The three common carrier gasses (helium, hydrogen, and nitrogen) can be compared by plotting carrier gas linear velocity against the height equivalent of a theoretical plate (HETP). An understanding of the relationship between carrier gas linear velocity and optimum efficiency can then be achieved. The modified Golay plot highlights some key qualities of each carrier gas.

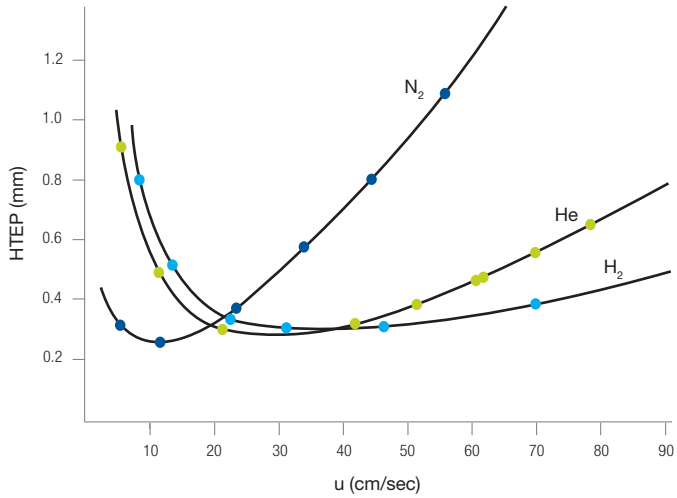

Figure 2. Golay plot of carrier gas HETP vs. linear velocity for helium, hydrogen, and nitrogen.

When comparing the modified Golay plot of helium (the most common carrier gas) to hydrogen, it can be seen that the highest efficiency separations (the minima in the plots) occur at similar linear velocities. However, as velocity increases, the increase in HETP, and therefore the corresponding drop in efficiency, is less pronounced with hydrogen. This property allows high linear velocity separations without a significant loss in resolution, making very fast analysis possible.

When comparing the modified Golay plot of helium to nitrogen, it can be seen that the highest efficiency separations (the plot minima) occur with nitrogen. This means that for a given column, the highest resolution of critical pairs in a chromatographic separation can be achieved with nitrogen. However, since the optimal linear velocity of nitrogen is significantly lower than helium and occurs over a very narrow range which drops off sharply, these high efficiency separations occur at the expense of analysis speed.

Instrument choice can also affect the analysis. The experiments performed here used the Thermo Scientific™ TRACE™ 1300 Series Gas Chromatograph, which is the latest technology to simplify workflow and increase analytical performance. The TRACE 1300 Series GC offers the most versatile GC platform in the market, with unique "Instant Connect" modularity for ground-breaking ease of use and performance, setting a new era in GC technology.

Detection was carried out on a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID) and data capture and analysis using Thermo Scientific™ Chromeleon™ 7.2 SR3 Chromatography Data System.

Experimental

Consumables

Column

- TRACE TR-FAME, 10 m × 0.1 mm × 0.2 µm (P/N 260M096P)

Injection septum

- Thermo Scientific™ BTO, 11 mm (P/N 31303233-BP)

Injection liner

- Thermo Scientific™ LinerGOLD™, Split/Splitless liner with glass wool (P/N 453A2265-UI)

Column ferrules

- 15% Graphite/85% Vespel® 0.1–0.25 mm (P/N 290VA191)

Injection syringe

- 10 µL fixed needle syringe for Thermo Scientific™ TriPlus™ RSH Autosampler (P/N 365D0291)

Vials and closures

- Thermo Scientific™ National™ SureStop™ MS Certified 9 mm screw vials with Blue Silicone/PTFE AVCS closure (P/N MSCERT5000-34W)

Compounds

A mixture containing the most common 37 FAMEs was used. Contents are detailed in Table 1.

Table 1. Summary table of components present within the 37 FAME standard.

Peak Name	Component*
Methyl butyrate	1
Methyl hexanoate	2
Methyl octanoate	3
Methyl decanoate	4
Methyl undecanoate	5
Methyl laurate	6
Methyl tridecanoate	7
Methyl myristate	8
Methyl myristoleate	9
Methyl pentadecanoate	10
Methyl *cis*-10-pentadecenoate	11
Methyl palmitate	12
Methyl palmitoleate	13
Methyl heptadecanoate	14
cis-10-Heptadecanoic acid methyl ester	15
Methyl stearate	16
trans-9-Elaidic acid methyl ester	17
cis-9-Oleic acid methyl ester	18
Methyl linolelaidate	19
Methyl linoleate	20
Methyl arachidate	21
Methyl γ-linolenate	22
Methyl *cis*-11-eicosenoate	23
Methyl linolenate	24
Methyl heneicosanoate	25
cis-11,14-Eicosadienoic acid methyl ester	26
Methyl behenate	27
cis-8,11,14-Eicosatrienoic acid methyl ester	28
Methyl erucate	29
cis-11,14,17-Eicosatrienoic acid methyl ester	30
cis-5,8,11,14-Eicosatetraenoic acid methyl ester	31
Methyl tricosanoate	32
cis-13,16-Docosadienoic acid methyl ester	33
Methyl lignocerate	34
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	35
Methyl nervonate	36
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	37

*Peaks were not identified by MS and were therefore only tentatively assigned.

Sample Pre-treatment

The test mix was injected as supplied without any dilution.

Method Optimization

Three carrier gases were investigated using the same instrumentation and column.

Instrumentation

- TRACE 1310 GC (P/N 14800302)
- TriPlus RSH Autosampler (P/N 1R77010-0100)
- Instant Connect Electron Flame Ionization Detector (FID) (P/N 19070001FS)

Separation Conditions

Experiment 1 (Helium)

Carrier Gas	Helium
Split Flow	88.0 mL/min
Split Ratio	251:1
Column Flow	0.35 mL/min
Oven Temperature	40 °C (1 min hold), 80 °C/min to 150 °C (0 min hold), 8 °C/min to 240 °C (1 min hold)
Injector Type	Split/Splitless
Injector Mode	Split, constant flow
Injector Temperature	220 °C
Detector Type	Flame ionization detector (FID)
Detector Temperature	250 °C
Detector Air Flow	350 mL/min
Detector Hydrogen Flow	35 mL/min
Detector Nitrogen Flow	40 mL/min

Experiment 2 (Hydrogen)

Carrier Gas	Hydrogen
Split Flow	75.0 mL/min
Split Ratio	250:1
Column Flow	0.30 mL/min
Oven Temperature	40 °C (0.83 min hold), 96 °C/min to 150 °C (0 min hold), 9.6 °C/min to 240 °C (0.2 min hold)
Injector Type	Split/Splitless
Injector Mode	Split, constant flow
Injector Temperature	220 °C
Detector Type	Flame ionization detector (FID)
Detector Temperature	250 °C
Detector Air Flow	350 mL/min
Detector Hydrogen Flow	35 mL/min
Detector Nitrogen Flow	40 mL/min

Experiment 3 (Nitrogen)

Carrier Gas	Nitrogen
Split Flow	28.0 ml/min
Split Ratio	255:1
Column Flow	0.11 mL/min
Oven Temperature	40 °C (2.07 min hold), 38.57 °C/min to 150 °C (0 min hold), 3.86 °C/min to 240 °C (0.62 min hold)
Injector Type	Split/Splitless
Injector Mode	Split, constant flow
Injector Temperature	220 °C
Detector Type	Flame ionization detector (FID)
Detector Temperature	250 °C
Detector Air Flow	350 mL/min
Detector Hydrogen Flow	35 mL/min
Detector Nitrogen Flow	40 mL/min

Data Processing

Software

Chromeleon 7.2 SR3 Chromatography Data System.

Results and Discussion

Typically, methods for FAME analysis have been carried out using a 100 m × 0.25 mm × 0.2 µm biscyanopropyl column with helium carrier gas. This required analysis times of around an hour to obtain the necessary resolution of the major components.

The equivalent separation on the 10 m length column with a narrower, 0.1 mm ID diameter is shown below (Figures 3a–c). By changing the column dimensions, the analysis time was reduced to approximately 12 minutes while maintaining resolution and efficiency.

In previously published methods, the components 25–32 were least resolved. Maintaining good separation of critical pairs in this region of the chromatogram was a key objective for this updated method. By using the 10 m column, the separation of critical pairs 25–26 and 28–29 was significantly improved compared to the 100 m column (Figures 3a–c). This is largely due to the increased efficiency of the narrower ID column.

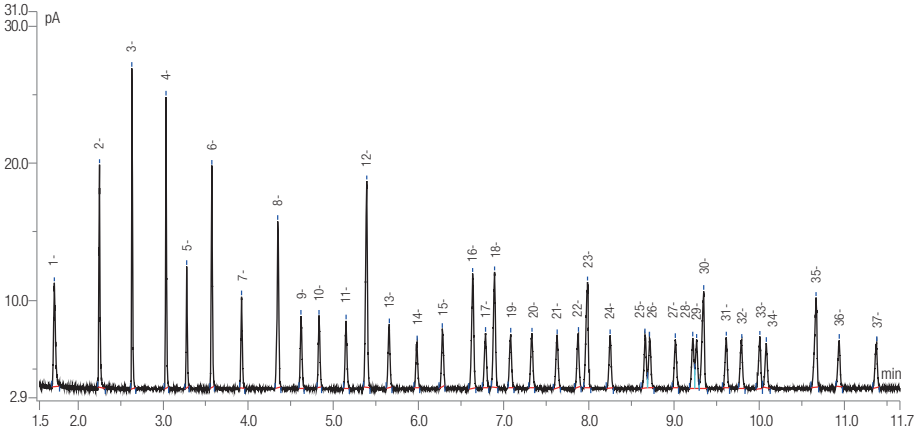

Figure 3a. Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with helium carrier gas. (Experiment 1)

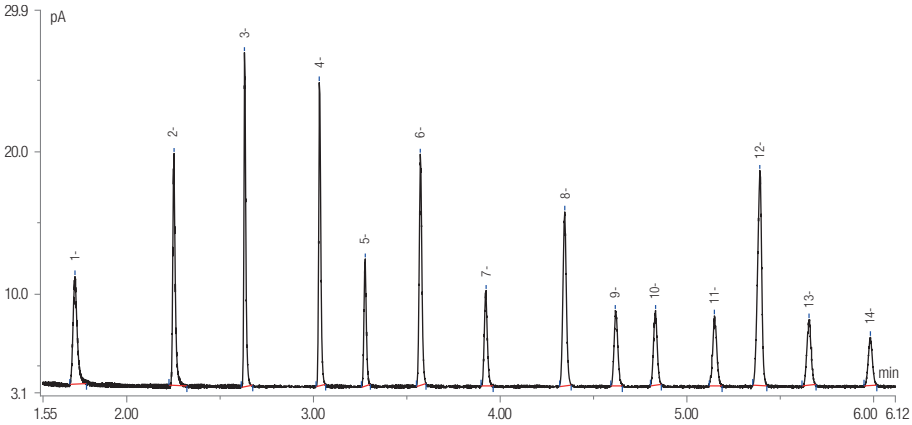

Figure 3b (peaks 1–14). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with helium carrier gas. (Experiment 1)

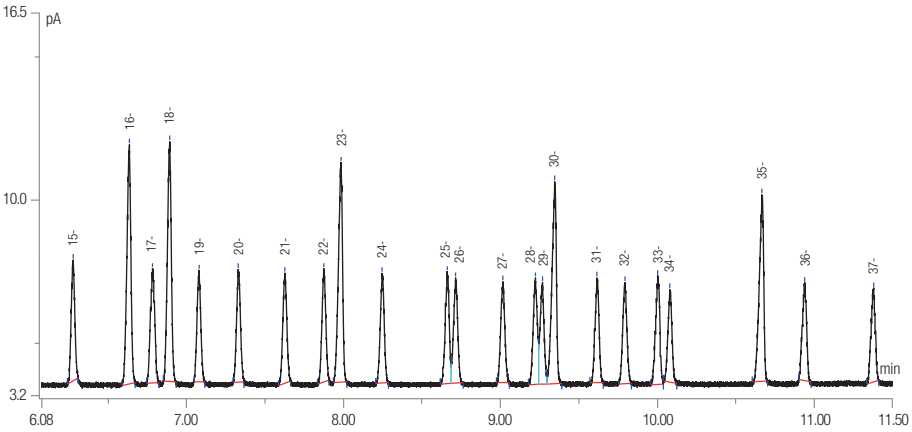

Figure 3c (peaks 15–37). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with helium carrier gas. (Experiment 1)

Conditions for the helium carrier gas separation were fully optimized and further improvements in speed or efficiency could only be achieved with this column using alternative carrier gasses. The next sets of experiments were conducted using hydrogen to attempt improvements in speed of analysis.

Hydrogen was able to give a faster separation than helium with all 37 components eluting in less than 9.3 minutes. There was, however, an impact on resolution of critical pairs (Figures 4a–c). While resolution was reduced, it was still possible to successfully integrate all peaks and for the majority, the resolution was still > 1.5 (Table 2).

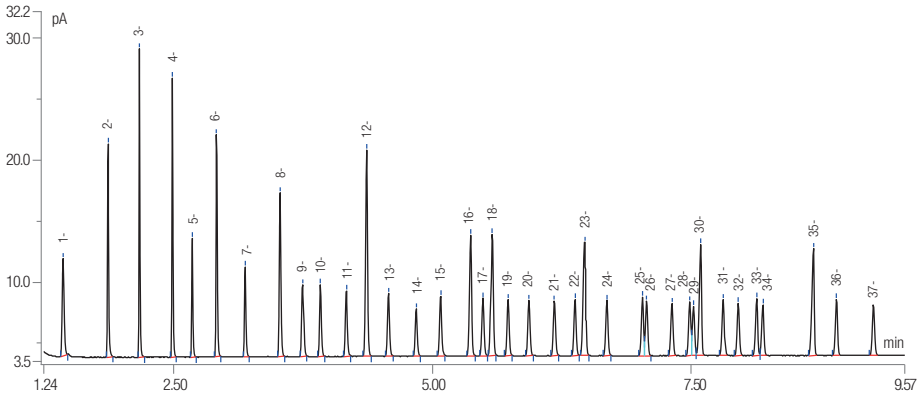

Figure 4a (full chromatogram hydrogen). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with Hydrogen carrier gas. (Experiment 2)

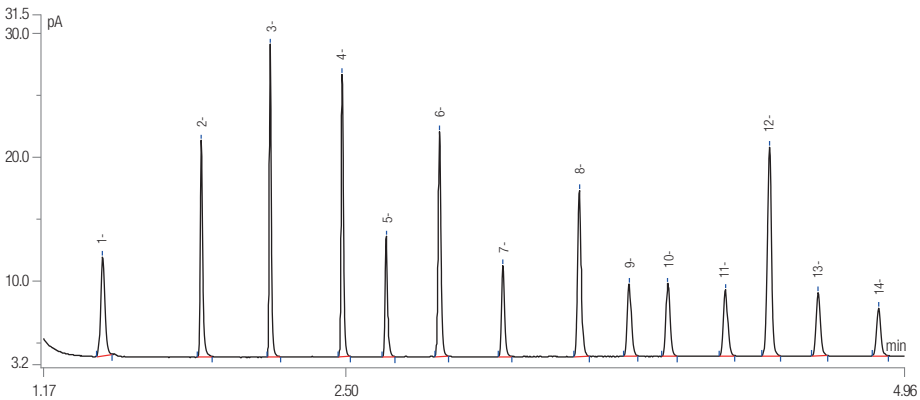

Figure 4b (peaks 1–14). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with hydrogen carrier gas. (Experiment 2)

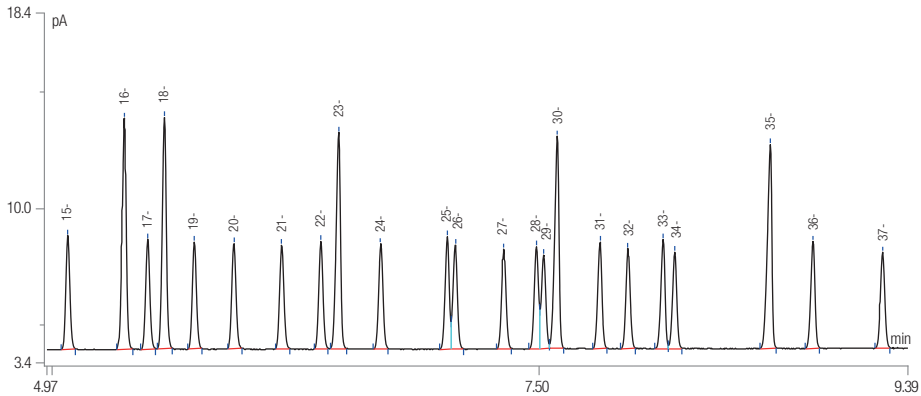

Figure 4c (peaks 15–37). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with hydrogen carrier gas. (Experiment 2)

Table 2. Resolution for all components.

Peak Name	Component*	Helium Resolution (EP)	Hydrogen Resolution (EP)	Nitrogen Resolution (EP)
Methyl butyrate	1	17.82	16.18	21.69
Methyl hexanoate	2	19.37	17.74	22.53
Methyl octanoate	3	21.46	19.83	24.28
Methyl decanoate	4	11.5	10.68	13.08
Methyl undecanoate	5	12.15	11.45	13.88
Methyl laurate	6	12.85	12.03	14.54
Methyl tridecanoate	7	13.49	12.57	15.27
Methyl myristate	8	7.75	7.28	8.92
Methyl myristoleate	9	5.94	5.41	6.59
Methyl pentadecanoate	10	8.62	7.73	9.47
Methyl *cis*-10-pentadecenoate	11	6.23	5.66	6.76
Methyl palmitate	12	6.52	6.07	7.31
Methyl palmitoleate	13	8.08	7.42	8.83
Methyl heptadecanoate	14	7.16	6.47	7.69
cis-10-Heptadecanoic acid methyl ester	15	8.04	7.54	8.82
Methyl stearate	16	3.18	3.08	3.67
trans-9-Elaidic acid methyl ester	17	2.33	2.2	2.62
cis-9-Oleic acid methyl ester	18	4.15	4.02	4.7
Methyl linolelaidate	19	5.52	5.13	6.15
Methyl linoleate	20	6.44	5.99	7.06
Methyl arachidate	21	5.41	4.99	5.78
Methyl γ-linolenate	22	2.33	2.29	2.48
Methyl *cis*-11-eicosenoate	23	5.61	5.26	6.15
Methyl linolenate	24	9.08	8.62	9.74
Methyl heneicosanoate	25	1.16	0.99	1.27
cis-11,14-Eicosadienoic acid methyl ester	26	6.38	5.87	7.07
Methyl behenate	27	4.19	3.94	4.71
cis-8,11,14-Eicosatrienoic acid methyl ester	28	0.92	0.85	1
Methyl erucate	29	1.63	1.66	1.81
cis-11,14,17-Eicosatrienoic acid methyl ester	30	5.52	5.43	6.09
cis-5,8,11,14-Eicosatetraenoic acid methyl ester	31	3.76	3.52	4.07
Methyl tricosanoate	32	4.49	4.48	4.86
cis-13,16-Docosadienoic acid methyl ester	33	1.66	1.54	1.79
Methyl lignocerate	34	11.87	11.88	13.1
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	35	5.51	5.33	6.03
Methyl nervonate	36	9.32	8.76	10.05
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	37	"	"	"

*Peaks were not identified by MS and were therefore only tentatively assigned.

The throughput of separations based on all three carrier gasses run times (Table 3) with 6-minute recycling time is given below (Figure 5). Published methods on 100 m columns using helium carrier gas could practically analyze up to 24 samples per day. Moving to a 10 m column increases throughput to a maximum of 80 samples per day. Even the use of a shorter column with nitrogen carrier gas increases throughput to 48 samples per day. If the carrier gas is then changed to hydrogen this further increases as high as 100 samples per day, a 400% increase.

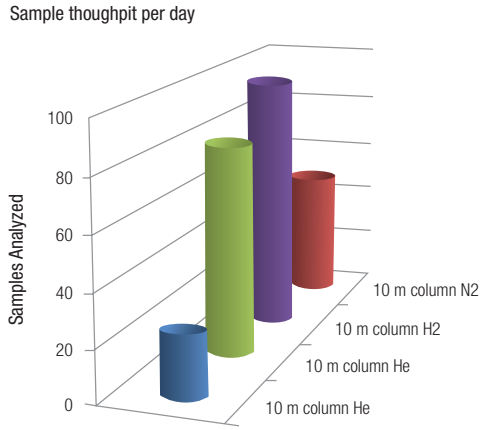

Figure 5. Sample throughput when comparing a 100 m column to a 10 m column using helium, hydrogen, and nitrogen as carrier gases.

Table 3. Experiment run times.

Experiment	Carrier gas	Run time (min)
1	Helium	11.9
2	Hydrogen	9.5
3	Nitrogen	23.7

Further experiments were then conducted using nitrogen in an attempt to increase separation efficiency and gain improvements in resolution. Figures 6a–c show the separation achieved.

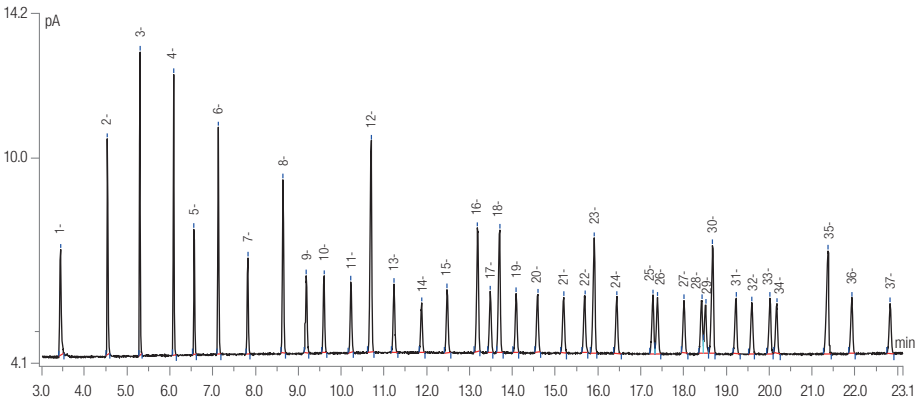

Figure 6a (full chromatogram nitrogen). Analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm cyanopropyl phase using nitrogen carrier gas. (Experiment 3)

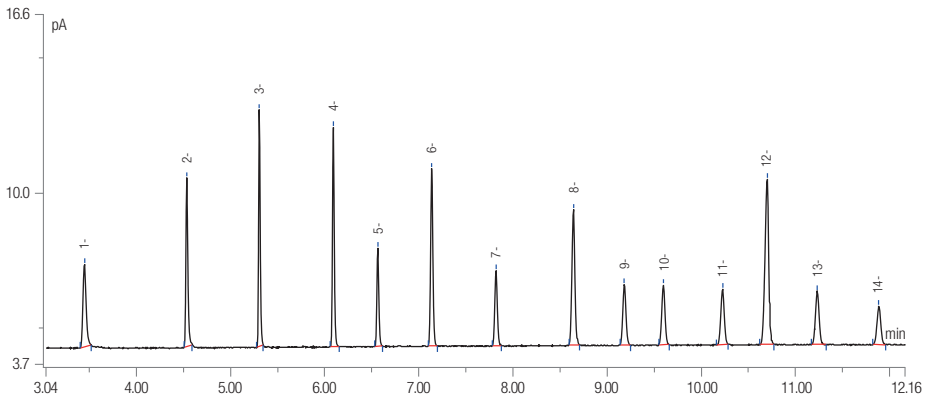

Figure 6b (peaks 1–14). Analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm cyanopropyl phase using nitrogen carrier gas. (Experiment 3)

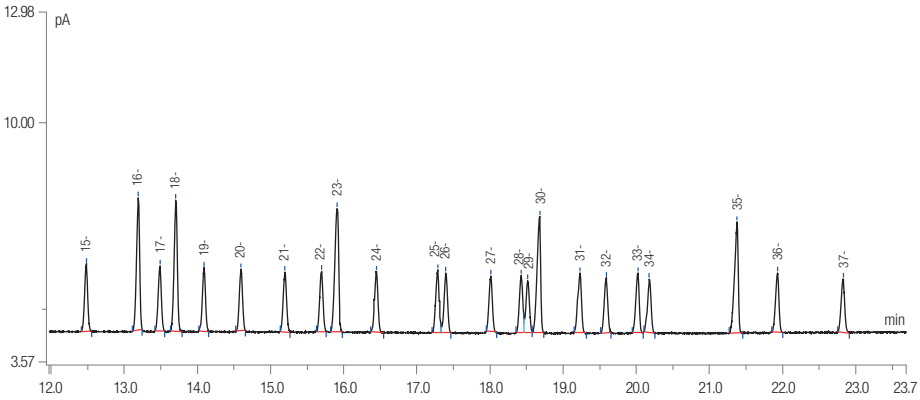

Figure 6c (peaks 15–37). Analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm cyanopropyl phase using nitrogen carrier gas. (Experiment 3)

The differences in resolution for all components using each carrier gas are displayed graphically (Figure 7), while individual resolution values are tabulated (Table 2).

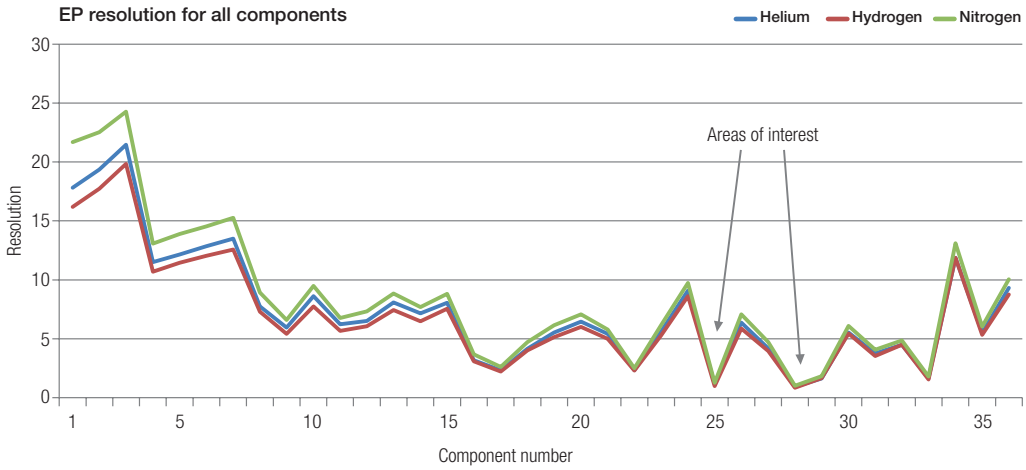

Figure 7. Graphs to show differences in carrier gas resolution for all components when comparing helium, hydrogen, and nitrogen.

In this graph it can be seen that resolution is greatest for nitrogen for all components, with the green line tracking highest across the range. For most peaks, there is significant resolution and the use of nitrogen as a carrier gas is not required; however, in the highlighted region, 25–26 and 28–29, it becomes crucial. The regions for these critical peaks were expanded to look closer at resolution differences between the different carrier gasses (Figure 8).

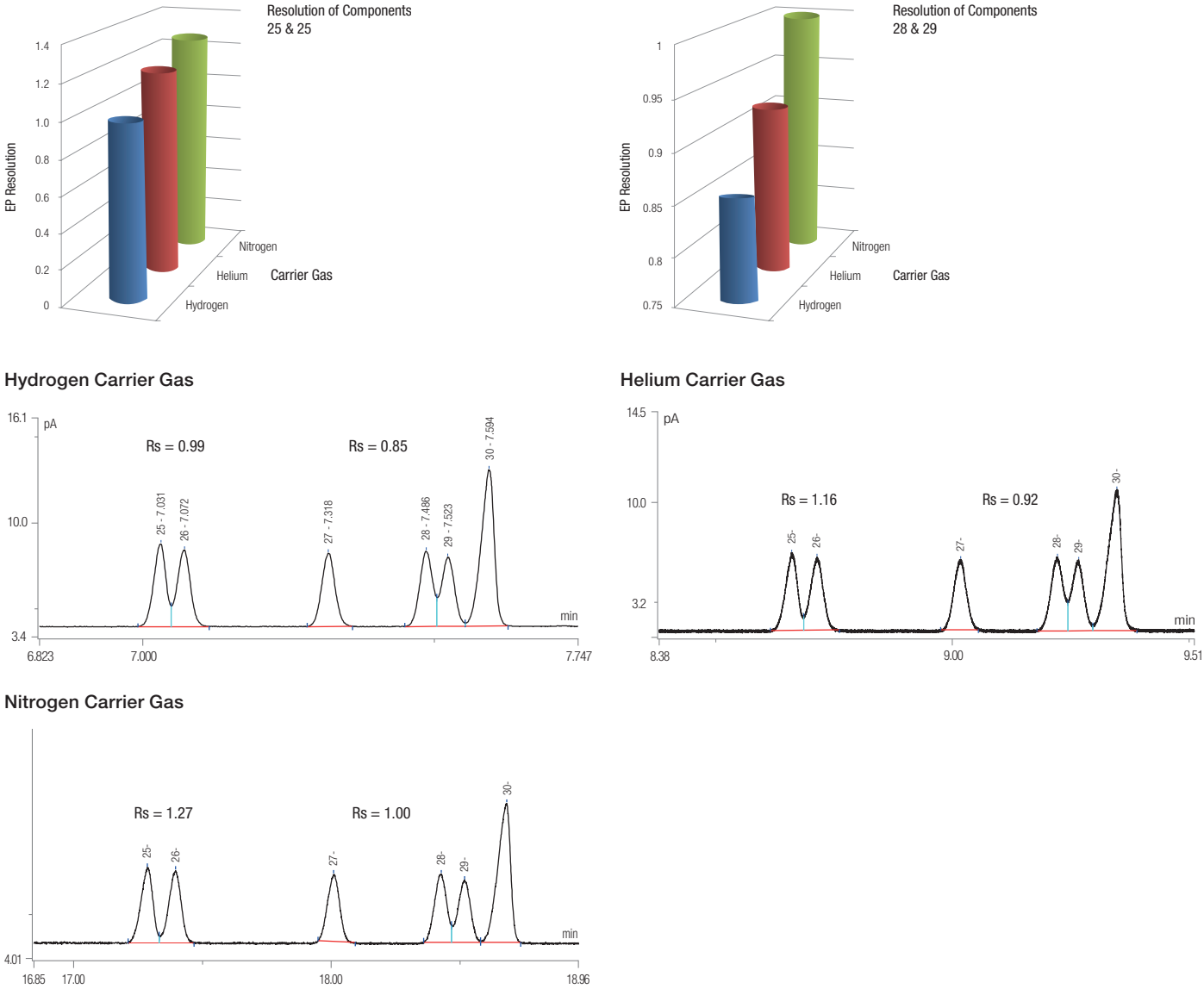

Figure 8. Graphs and chromatograms to show differences in carrier gas resolution for critical pairs when comparing hydrogen, helium, and nitrogen.

As seen above, the separation of the critical pairs is better with the nitrogen carrier gas. The resolution of critical pairs 25–26 and 28–29 was significantly improved compared with separations using helium and hydrogen. Resolution for peaks 25–26 for the nitrogen carrier gas was found to be 22% greater than hydrogen and 9% greater than helium. Similarly comparing the resolution for peaks 28–29 using nitrogen carrier gas was found to be 15% greater than hydrogen and 8% greater than helium.

Due to the increased efficiency of nitrogen as a carrier gas, the critical components could be better resolved. The benefit of an increase in resolution includes improvement in quantitation as peak assignment and integration are both easier to achieve. This translates to improved confidence in the results and the achievement of lower detection levels.

An analysis of the increased resolution found for nitrogen revealed increased peak efficiencies of the critical pairs, compared to the other carrier gasses. EP plate count (a standard measure of efficiency) was used to determine this (Table 4).

Nitrogen was found to be 18% more efficient than helium and 39% more efficient than hydrogen under these conditions. The efficiency gain meant that peak resolution was significantly improved to the hydrogen.

Table 4. Efficiencies of different carrier gases.

Peak Number	Peak Name	Helium	Hydrogen	Nitrogen	Efficiency Increase %	
		Plates			N_2 compared to HE	N_2 compared to H
25	Component 25	573023	505099	642339	12	27
26	Component 26	530880	451019	639285	20	42
27	Component 27	536955	472868	659995	23	40
28	Component 28	596524	470381	690580	16	47
				Mean %	18	39

Conclusions

- The separation of 37 fatty acid methyl esters (FAMEs) on the highly efficient 10 m TR-FAME GC column was significantly improved compared to the analysis on a 100 m FAMEs column, demonstrating greater resolution and increased sample throughput of up to 400%.

- By using different carrier gases, the separation of FAMEs can be optimized for reduced analysis time, resolution of critical pairs, and efficiency.

Find out more at **thermofisher.com/columnsforgc**

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