Automated Standard and Sample Preparation Using the Agilent 7696A WorkBench for GC/MS Analysis of FAME Contamination in Jet Fuel

Application Note

Fuels

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Abstract

The Agilent 7696A Sample Prep WorkBench was used to prepare calibration standards and samples for the GC/MS analysis of total FAME in jet fuel using the IP585 method. The WorkBench needed 10 times less reagents and standards to achieve better analysis results when compared to manual sample preparation techniques. The GC/MS calibration using WorkBench prepared standards meet all performance criteria without any re-work, saving considerable time in the laboratory. WorkBench prepared jet fuel samples exceeded the method's precision requirements for several different levels of FAME contamination. The analysis results obtained from the WorkBench samples provided better recovery of the known FAME concentrations compared to the manually prepared samples.

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Introduction

The Energy Institute method, IP585, uses GC/MS to measure trace fatty acid methyl esters (FAME) in commercial jet fuel.[1] FAME contamination occurs when multiproduct pipelines (MPP) are used to transport both biodiesel and jet fuel. A limit of 5 mg/kg of total FAME content has been established by the Joint Inspection Group (JIG), a consortium of jet fuel producers and users. A recent Agilent paper describes the operation and performance of the Agilent 5975C Series GC/MSD system when running method IP585.[2]

As with most instrumental measurements, successful preparation of calibration standards and samples plays a significant part to achieving quality results. For the IP585 method, 1-mL volumes of calibration standards are made using graduated microliter pipettes. Using a microliter syringe, an expensive internal standard solution containing 1000 mg/mL of methyl heptadecanoate-d33 (C17:0-d33) is added to every calibration standard and sample. Due to the small volumes being measured, these procedures require considerable skill to correctly prepare standards and samples. A better approach would be to automate the sample preparation using an instrument specifically designed to dispense and mix liquids in microliter volumes with high accuracy and precision.

The Agilent 7696A Sample Prep WorkBench is a standalone instrument specifically designed to perform automated sample preparation. It uses two Agilent 7693A injection towers to volumetrically transfer liquids between 2-mL vials. Vials containing various chemical resources, standards, and samples are housed in three 50-positions trays. The sample tray compartment contains a robotic arm, a vortex mixing station, and a sample heating station. Calibration standard preparation using the Agilent WorkBench have been shown to provide better calibrations compared to manually prepared

standards. Additionally, samples prepared in 2-mL vials using the WorkBench were shown to give the same quantitative results as manually prepared samples.[3] In this application note, the Agilent 7696A Workbench was used to prepare 11 calibration standards along with three jet fuel samples each containing different levels of FAME contamination. Standards and sample volumes were reduced 10-fold from 1 mL to 100 µL to save resources such as solvents, stock standard solutions and the internal standard solution. The analysis results from the WorkBench prep were compared to results from a manual prep using the precision specifications in the IP585 method.

Designing the Automated Workbench Procedure

Calibration Standards Prepared by Linear Dilution

The IP585 method uses 10 working calibration standards (WCS) to calibrate the GC/MS system. Each WCS contains different concentrations of the six FAMEs shown in Table 1. The linear dilution scheme outlined in Table 2 is described in the method to manually prepare 1 mL quantities of each WCS. For the automated WorkBench preparation, this manual scheme was translated from 1 mL to 100 µL final volumes for each standard as shown in Table 3. To prepare the standards, four resources were defined in the WorkBench software (Table 4). The first resource was 10 empty vials used to contain the final WCS. The next resource was a vial containing 1,000 µL of 99% n-dodecane used as the dilution solvent. The third resource was a vial containing 1,000 µL of the working standard solution (WSS). The last resource was a vial containing 500 µL of the internal standard solution. Figure 1 shows the resource layout used by the WorkBench software for automated preparation of the calibration standards.

Table 1.   Compounds used to Quantify Total FAME in Jet Fuel

Chemical name	Common name	Symbol	Molecular formula	Molecular weight
Methyl hexadecanoate	Methyl palmitate	C16:0	$C_{17}H_{34}O_2$	270.45
Methyl heptadecanoate	Methyl margarate	C17:0	$C_{18}H_{36}O_2$	284.45
Methyl octadecanoate	Methyl stearate	C18:0	$C_{19}H_{38}O_2$	298.50
Methyl octadecenoate	Methyl oleate	C18:1	$C_{19}H_{36}O_2$	296.49
Methyl octadecadienaote	Methyl linoleate	C18:2	$C_{19}H_{34}O_2$	294.47
Methyl octadecatrienoate	Methyl linolenate	C18:3	$C_{19}H_{32}O_2$	292.45

These six FAMEs are found in 95% of the common feed stocks used to produce biodiesel.

Table 2.   Manual Scheme to Prepare 1-mL of each Working Calibration Standard (WCS) using Linear Volumetric Dilution

Volume (µL) of working standard solution (WSS)	Volume (µL) of n-C12 solvent	Volume (µL) of internal standard (ISTD)	Final concentration (mg/kg) of each FAME
1000	0	10	100
800	200	10	80
600	400	10	60
400	600	10	40
200	800	10	20
100	900	10	10
80	920	10	8
60	940	10	6
40	960	10	4
20	980	10	2
0	1000	10	0

Table 3.   Agilent WorkBench Linear Volumetric Dilution Preparation to Make 100 µL of each Working Calibration Standard (WCS)

Volume (µL) of working standard solution (WSS)	Volume (µL) of n-C12 solvent	Volume (µL) of internal standard (ISTD)	Final concentration (mg/kg) of each FAME	Working calibration standards (WCS)
100	0	1	100	High Std 5
80	20	1	80	High Std 4
60	40	1	60	High Std 3
40	60	1	40	High Std 2
20	80	1	20	High Std 1
10	90	1	10	Low Std 5
8	92	1	8	Low Std 4
6	94	1	6	Low Std 3
4	96	1	4	Low Std 2
2	98	1	2	Low Std 1
0	100	1	0	Blank

Table 4.   WorkBench Resource Layout for Automated Preparation of IP585 Calibration Standards

Resource	Resource type	Vial range	Usage
Working calibration standards (WCS)	Empty container	51-60	1
n-Dodecane solvent	Chemical resource	61	1000 µL
Working standard solution (WSS)	Chemical resource	71	1000 µL
Internal standard solution (ISTD)	Chemical resource	81	500 µL
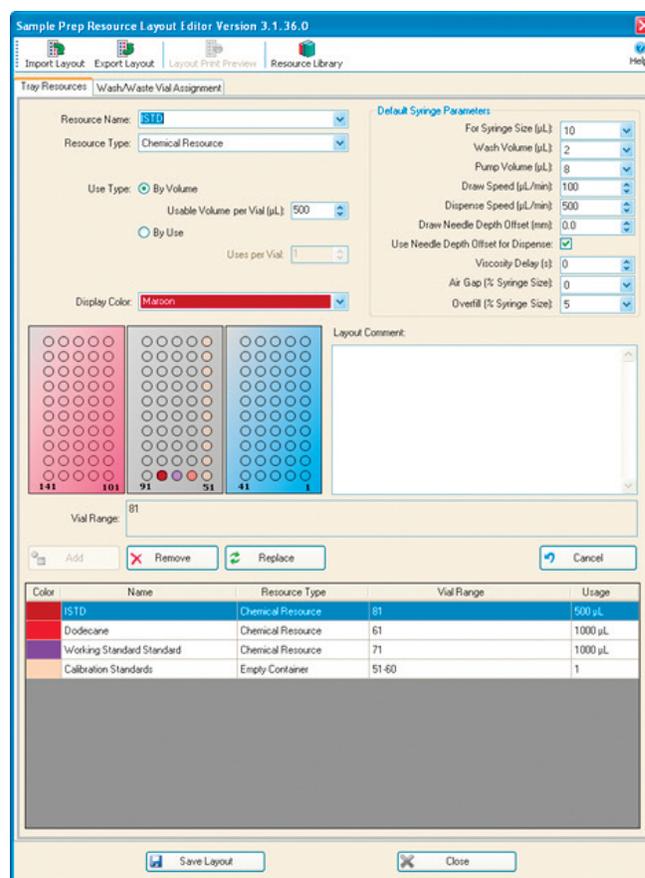
Figure 1.   Agilent WorkBench resource layout for the automated preparation of IP585 calibration standards. The empty vials in positions 51 to 60 will contain each of the 10 calibration standards after the automated preparation is complete.

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With the resource layout complete, two Agilent WorkBench methods were designed to prepare the standards listed in Table 3. The first method, "IP585_Low.M", was used for the 2 to 10 mg/kg low level standards and the second method, "IP585_High.M", was used for the 20 to 100 mg/kg high level standards. Details of the sample prep steps for each of these methods are listed in Tables 5 and 6. The WorkBench software allows the user to quickly and easily build methods using a graphical "drag-and-drop" interface. The IP585_Low.M method shown in Figure 2 is an example of a typical method.

Table 5. *Agilent WorkBench Method to Prepare 100 µL of each Low Level Working Calibration Standard (WCS)*

Step	Agilent WorkBench action	Description	Syringe
1	Wash	Solvent wash 250 µL syringe	250 µL
2	Add	100 µL n-C_{12} to Low Blank (Vial 1)	250 µL
3	Add	98 µL n-C_{12} to Low Std 1 (Vial 51)	250 µL
4	Add	96 µL n-C_{12} to Low Std 2 (Vial 52)	250 µL
5	Add	94 µL n-C_{12} to Low Std 3 (Vial 53)	250 µL
6	Add	92 µL n-C_{12} to Low Std 4 (Vial 54)	250 µL
7	Add	90 µL n-C_{12} to Low Std 5 (Vial 55)	250 µL
8	Wash	Solvent wash 25 µL syringe	25 µL
9	Add	2 µL WSS to Low Std 1 (Vial 51)	25 µL
10	Add	4 µL WSS to Low Std 2 (Vial 52)	25 µL
11	Add	6 µL WSS to Low Std 3 (Vial 53)	25 µL
12	Add	8 µL WSS to Low Std 4 (Vial 54)	25 µL
13	Add	10 µL WSS to Low Std 5 (Vial 55)	25 µL
14	Wash	Solvent wash 25 µL syringe	25 µL
15	Add	1 µL ISTD to Low Blank (Vial 1)	25 µL
16	Add	1 µL ISTD to Low Std 1 (Vial 51)	25 µL
17	Add	1 µL ISTD to Low Std 2 (Vial 52)	25 µL
18	Add	1 µL ISTD to Low Std 3 (Vial 53)	25 µL
19	Add	1 µL ISTD to Low Std 4 (Vial 54)	25 µL
20	Add	1 µL ISTD to Low Std 5 (Vial 55)	25 µL
21	Wash	Solvent wash 25 µL syringe	25 µL
22	Mix	Low Blank (Vial 1) for 30 s @ 1500 rpm	
23	Mix	Low Std 1 (Vial 51) for 30 s @ 1500 rpm	
24	Mix	Low Std 2 (Vial 52) for 30 s @ 1500 rpm	
25	Mix	Low Std 3 (Vial 53) for 30 s @ 1500 rpm	
26	Mix	Low Std 4 (Vial 54) for 30 s @ 1500 rpm	
27	Mix	Low Std 5 (Vial 55) for 30 s @ 1500 rpm	

Table 6. *Agilent WorkBench Method to Prepare 100 µL of each High Level Working Calibration Standard (WCS)*

Step	Agilent WorkBench action	Description	Syringe
1	Wash	Solvent wash 250 µL syringe	250 µL
2	Add	100 µL n-C_{12} to High Blank (Vial 2)	250 µL
3	Add	80 µL n-C_{12} to High Std 1 (Vial 56)	250 µL
4	Add	60 µL n-C_{12} to High Std 2 (Vial 57)	250 µL
5	Add	40 µL n-C_{12} to High Std 3 (Vial 58)	250 µL
6	Add	20 µL n-C_{12} to High Std 4 (Vial 59)	250 µL
7	Wash	Solvent wash 250 µL syringe	250 µL
8	Add	20 µL WSS to High Std 1 (Vial 56)	250 µL
9	Add	40 µL WSS to High Std 2 (Vial 57)	250 µL
10	Add	60 µL WSS to High Std 3 (Vial 58)	250 µL
11	Add	80 µL WSS to High Std 4 (Vial 59)	250 µL
12	Add	100 µL WSS to High Std 5 (Vial 60)	250 µL
13	Wash	Solvent wash 25 µL syringe	25 µL
14	Add	1 µL ISTD to High Blank (Vial 2)	25 µL
15	Add	1 µL ISTD to High Std 1 (Vial 56)	25 µL
16	Add	1 µL ISTD to High Std 2 (Vial 57)	25 µL
17	Add	1 µL ISTD to High Std 3 (Vial 58)	25 µL
18	Add	1 µL ISTD to High Std 4 (Vial 59)	25 µL
19	Add	1 µL ISTD to High Std 5 (Vial 60)	25 µL
20	Wash	Solvent wash 25 µL syringe	25 µL
21	Mix	High Blank (Vial 2) for 30 s @ 1500 rpm	
22	Mix	High Std 1 (Vial 56) for 30 s @ 1500 rpm	
23	Mix	High Std 2 (Vial 57) for 30 s @ 1500 rpm	
24	Mix	High Std 3 (Vial 58) for 30 s @ 1500 rpm	
25	Mix	High Std 4 (Vial 59) for 30 s @ 1500 rpm	
26	Mix	High Std 5 (Vial 60) for 30 s @ 1500 rpm	
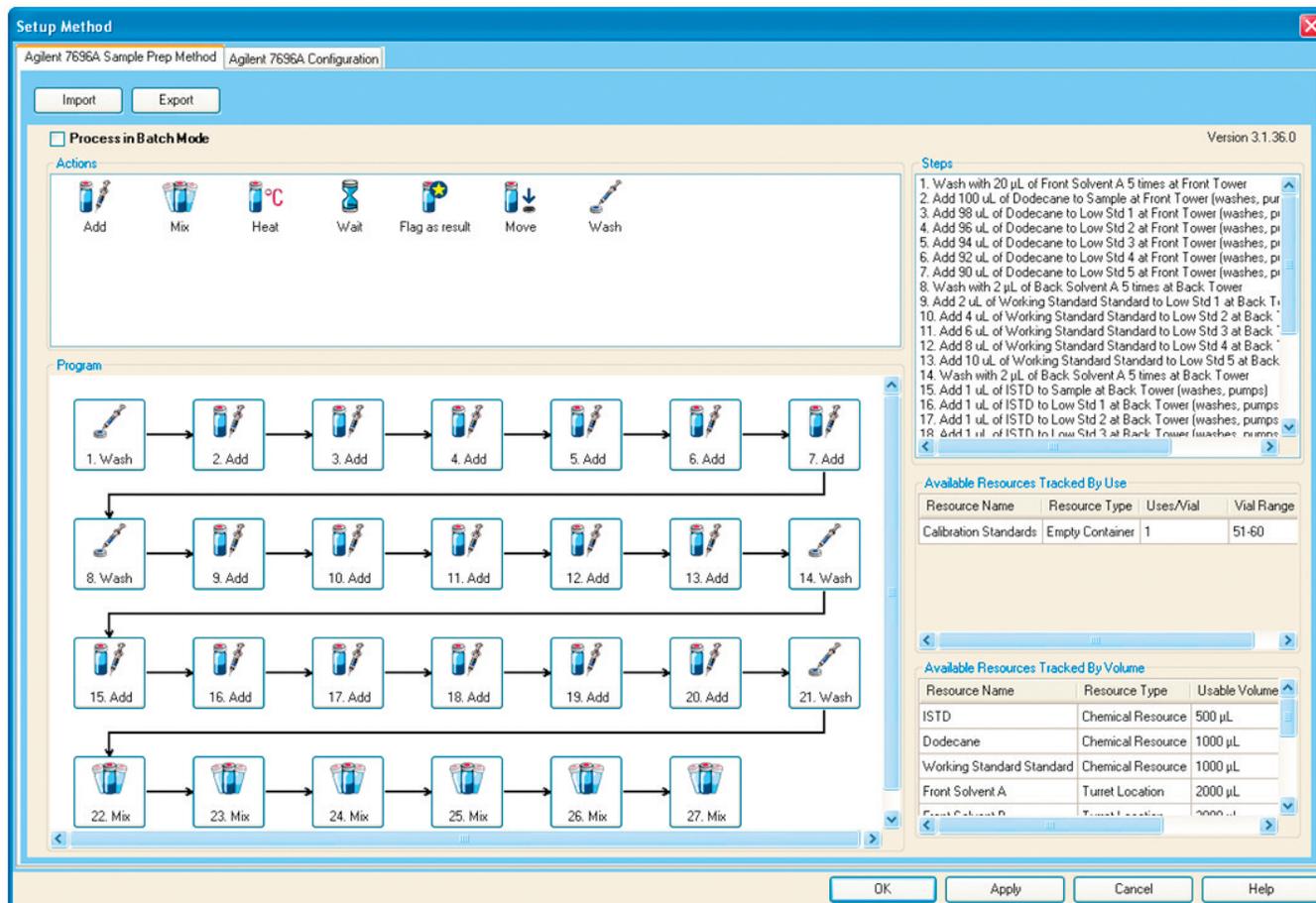
Figure 2.   Agilent WorkBench method IP585_Low.M for preparing five low level calibration standards. Each of the method's steps were built using a "drop-and-drag" graphic ser interface.

Jet Fuel Sample Preparation Using Agilent Workbench Batch Mode

For the IP585 method, samples were prepared by pipetting 1 mL of jet fuel into a 2-mL vial followed by the addition of 10 µL of the internal standard solution. A laboratory chemist manually preparing multiple samples performs a workflow by adding each jet fuel sample into individual vials followed by adding the internal standard to each sample. This efficient workflow can be performed by using the Batch Mode feature of the Agilent WorkBench software. In Batch Mode, each sample preparation step was completed for every sample before moving on to the next step so that sample preparation time was minimized. Solvent wash and waste resources are also conserved since syringe solvent washing is only needed between resource changes.

For jet fuel sample preparation, the WorkBench needs only two resources; vials containing each jet fuel sample and a single vial containing the internal standard solution. In this application note, ten separate jet fuel samples were defined as resources for the WorkBench. These vials were placed in tray positions 51 to 60 and usage was set to one use per vial to eliminate any possibility of cross contamination during preparation. The internal standard vial was placed in tray position 81. During the sample preparation runs, 10 empty and capped 2-mL vials were placed in tray positions 1 to 10 (Figure 3). The batch mode WorkBench method, IP585_Samples.M, dispensed 100 uL of each jet fuel sample into separate, empty vials, followed by the addition of 1 µL of internal standard solution and mixing. Figure 4 shows this batch mode method for the jet fuel sample preparation.

Experimental

Manual Preparation of Working Calibration Standards (WCS) and Samples

Following the procedure described in the method (Table 2), the 10 calibration standards and a solvent blank were manually prepared in 2-mL vials using 1,000 µL graduated pipettes and a 25 µL pipetting syringe. Manual sample preparation was done by pipetting 1 mL each of three different jet fuel samples into individual 2-mL vials followed by addition of 10 µL of the internal standard. These samples contained known amounts of total FAME and were prepared in duplicate to determine overall repeatability. Each standard and sample was manually shaken to assure mixing.
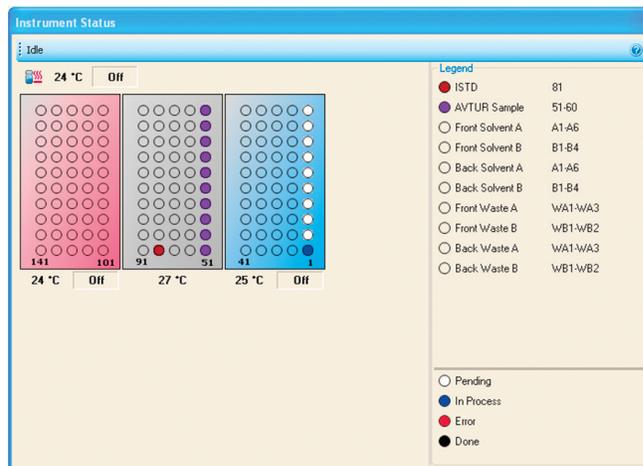
Figure 3.   Agilent WorkBench resource layout for the automated preparation of 10 jet fuel samples. The empty vials in positions 1 to 10 will contain the final 100 mL of each jet fuel sample and internal standard after the automated preparation is complete.
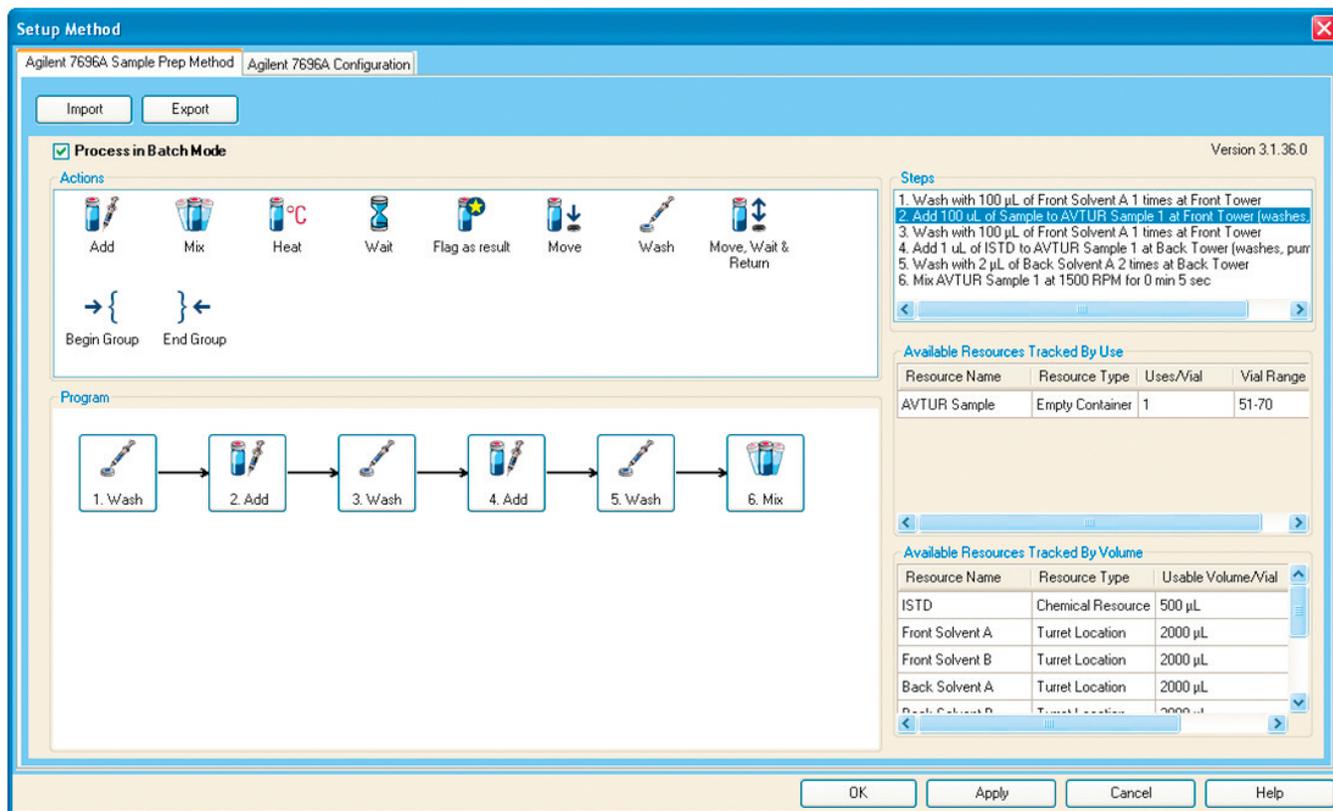
Figure 4.   Batch mode Agilent WorkBench method for preparing 10 jet fuel samples. Each step was performed for all 10 samples before moving onto the next step. This efficient workflow minimized time and resource usage.

Automated Preparation of Calibration Standards and Jet Fuel Samples

The Agilent WorkBench was configured with a 250 µL syringe in the front tower and a 25 µL syringe in the rear tower. The 250 µL syringe used a draw speed of 500 µL/min and a dispense speed of 1000 µL/min. A draw speed of 100 µL/min and dispense speed of 500 µL/min was used for the 25 µL syringe. For each syringe, the dispense depth was set to 0 mm so the needle was close to the bottom of the vial when dispensing liquids. This ensured complete transfer of the liquid into the vial resulting in the best possible precision. High recovery vials were used because the internal v-shape allows the GC/MS autosampler to have access to the small 100 µL volumes of standards and samples.

The WorkBench sequence queue was used to prepare 5 low level standards and 5 high level standards using the IP585_Low.M and the IP585_High.M methods. After GC/MS calibration verification, the WorkBench batch mode method, IP585_Samples.M, was used to prepare duplicates of the three jet fuels samples spiked with different amounts of FAME.

GC/MS Analysis of FAME in Jet Fuel

An Agilent 5975C GC/MS system with an Agilent 7693A Automated Liquid Sampler was configured according to the IP585 method. This configuration is described in Table 7 and the instrument operating conditions are shown in Table 8. The mass spectrometer was tuned using the Agilent 5975C Autotune program before running any standards or samples. The calibration standards and the n-dodecane solvent blank were run first and the linear performance of the low level calibration and the high level calibration were evaluated before running the jet fuel samples. Upon successful calibration, a single GC/MS analysis of each jet fuel sample duplicate was made. The individual FAME peaks were quantified and the total FAME content in each sample was calculated by summing the individual FAME results.

Table 7.   Instrument Configuration for GC/MS Analysis of FAMEs in Jet Fuel

Component	Description
Agilent 5975C Series MSD	Mass spectrometer with inert electron ionization source
Agilent 7890A GC system	Gas Chromatograph with 100 psi split/splitless inlet and mass spectrometer interface
Agilent 7693A ALS	Automatic liquid injector for Agilent 7890A GC with 150-vial tray
G1701EA	MSD Chemstation Software for data acquisition and analysis

Table 8.   GC/MS Instrument Conditions

GC conditions

Inlet temperature	260 °C
Inlet mode	Splitless
Inlet liner	Splitless liner, single taper glass wool (p/n 5062-3587)
Sample volume	1 uL
Column	HP-INNOWAX, 50 m x 0.2 mm, 0.4 µm film (p/n 19091N-205)
Column flow	Helium at 0.6 mL/min constant flow
Oven program	
Initial temperature	150 °C for 5 min
Oven ramp no 1	12 °C /min to 200 °C for 17 min
Oven ramp no 2	3 °C/min to 252 °C for 6.5 min
Mass spec interface	260 °C

Mass Spec Conditions

Ionization source	70 eV electron ionization
Source temperature	230 °C
Quadrupole temperature	150 °C
Data acquisition delay	20 min

Results

Comparison of Manual and Agilent WorkBench Calibration Performance

The calibration standards from both the manual and the Agilent WorkBench preparations were run on the Agilent 5975C GC/MS system. The individual FAME calibration curves resulting from the low and high level WorkBench standards are shown in Figures 5 and 6. All of these curves appear to be linear after regression analyses with the origins forced through 0. Comparisons of the manual and WorkBench calibrations are shown in Table 9. For the low level calibrations, the slopes of the manual and WorkBench calibrations are very similar and the correlation coefficients (R^2) all meet the method requirement of greater than 0.985. The high level calibrations show the same performance with the exception of the methyl linoleate (C18:2) and methyl linolenate (C18:3) calibrations. In this case, the WorkBench prepared standards easily met the method requirements, while the manually prepared standards failed the linearity test. Therefore the manually prepared jet fuel samples could not be run until the high level standards were remade and the calibrations correctly verified. This added considerable time in obtaining results for the manually prepared samples. However, since the WorkBench calibrations were initially correct, the WorkBench prepared jet fuel samples could be run immediately.
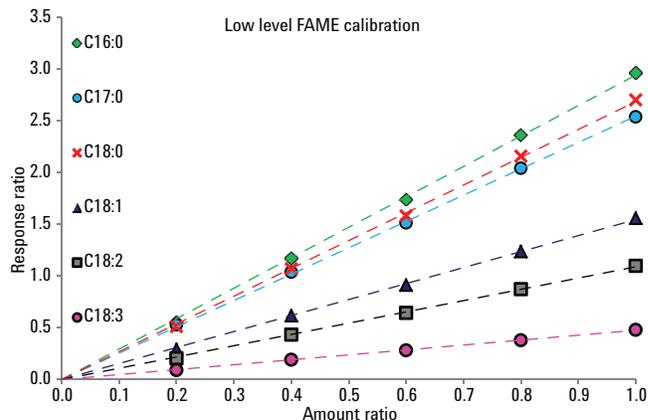
Figure 5. Low level calibration curves for 2, 4, 6, 8, and 10 mg/kg FAME standards prepared using the Agilent WorkBench. The calibration curves were forced through zero according to the method's protocol. Each curves exceeded the method's linearity requirement of $R^2 > 0.985$.
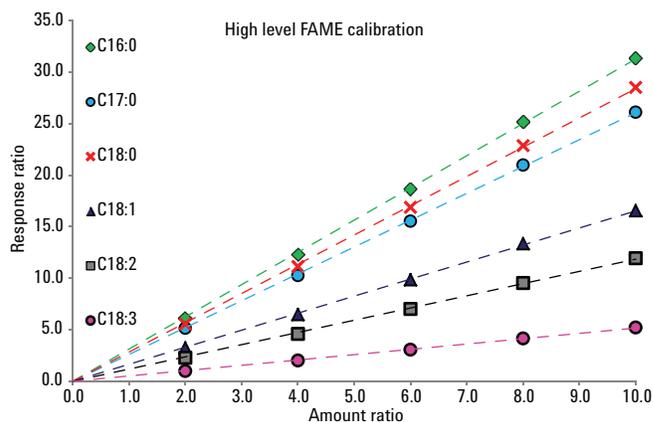
Figure 6. High level calibration curves for 20, 40, 60, 80, and 100 mg/kg FAME standards prepared using the Agilent WorkBench. The calibration curves were forced through zero according to the method's protocol. Each curve exceeded the method's linearity requirement of $R^2 > 0.985$.

Table 9. Comparison of the Slopes and Correlation Coefficients (R^2) Determined for Calibration Curves made using Manual and Agilent WorkBench Prepared Standards

Low Level Calibration (2–10 mg/kg)

FAME	Slope		R^2	
	Manual	WorkBench	Manual	WorkBench
C16:0	2.941	2.941	1.000	0.999
C17:0	2.441	2.544	1.000	1.000
C18:0	2.664	2.684	1.000	0.999
C18:1	1.539	1.545	1.000	0.999
C18:2	1.105	1.090	1.000	0.999
C18:3	0.478	0.475	1.000	0.999

High Level Calibration (20–100 mg/kg)

FAME	Slope		R^2	
	Manual	WorkBench	Manual	WorkBench
C16:0	4.962	3.127	0.985	1.000
C17:0	4.777	2.606	0.985	1.000
C18:0	4.815	2.840	0.985	1.000
C18:1	2.510	1.653	0.985	1.000
C18:2	1.713	1.184	0.984	0.999
C18:3	0.705	0.516	0.983	0.999

The manual high level calibrations curves for the C18:2 and C18:3 FAMEs failed the minimum R^2 requirement of 0.985.
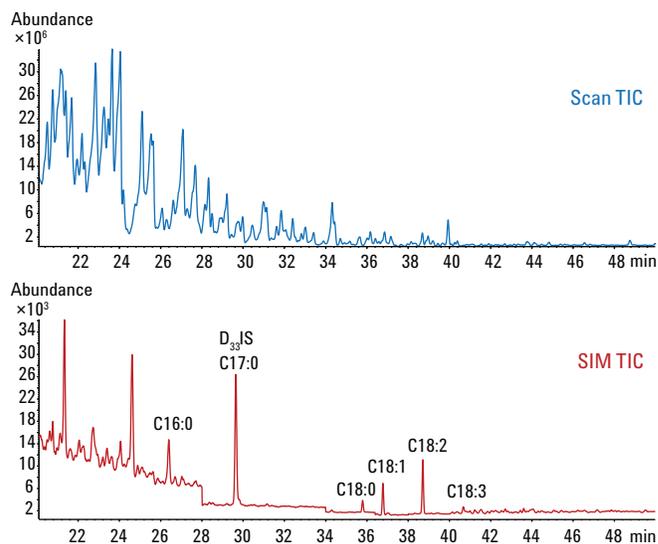
Figure 7. SIM/SCAN GC/MS data obtained from an Agilent WorkBench prepared jet fuel sample containing 5 mg/kg total FAME.

Comparison of Manual and Agilent WorkBench Sample Preparation

A typical GC/MS SIM/SCAN chromatogram for a jet fuel FAME analysis is shown in Figure 7. Comparisons of the analysis results for the manually prepared and the Agilent WorkBench prepared jet fuels are shown in Tables 10, 11, and 12. For each sample duplicate, repeatability (r) was calculated for the total FAME content and compared to the specification published in the IP585 method. Repeatability is a measurement of precision calculated by taking the difference between two duplicate results obtained on the same sample, by the same operator, using the same instrument, on the same day. For the 5 mg/kg FAME spike (Table 11), the repeatability of the manually prepared samples does not meet the IP585 method specification. Therefore, this result is invalid. However, for all WorkBench samples, the repeatabilities were much better than the method's specifications. Additionally, the results obtained with the Workbench samples more closely matched the total FAME content spiked into the jet fuel samples.

Table 10. Comparison of Analysis Results from a Manual and Agilent WorkBench Samples Preps for a 1 mg/kg FAME Jet Fuel Spike

1 mg/kg Jet fuel spike - Manual prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	0.8	0.0	0.1	0.3	0.1	0.0	1.3
Run 2	0.8	0.0	0.1	0.3	0.1	0.0	1.3
						Avg	1.3
						r (calc)	0.0
						r (IP585)	0.7

1 mg/kg Jet fuel spike - Agilent WorkBench prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	0.8	0.0	0.1	0.3	0.1	0.0	1.3
Run 2	0.7	0.0	0.1	0.3	0.1	0.0	1.2
						Avg	1.3
						r (calc)	0.1
						r (IP585)	0.7

Table 11. Comparison of Analysis Results from a Manual and Agilent WorkBench Samples Preps for a 5 mg/kg FAME Jet Fuel Spike

5 mg/kg Jet fuel spike - Manual prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	1.1	0.0	0.3	0.4	3.8	1.2	6.8
Run 2	0.5	0.0	0.2	0.9	2.6	0.7	4.9
						Avg	5.9
						r (calc)	1.9
						r (IP585)	1.4

5 mg/kg Jet fuel spike - Agilent WorkBench prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	0.5	0.0	0.1	0.9	2.7	0.5	4.7
Run 2	0.6	0.0	0.2	0.9	2.7	0.6	5.0
						Avg	4.9
						r (calc)	0.3
						r (IP585)	1.3

Table 12. Comparison of Analysis Results from a Manual and Agilent WorkBench Samples Preps for a 40 mg/kg FAME Jet Fuel Spike

40 mg/kg Jet fuel spike - Manual prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	4.4	0.0	1.7	7.9	24.0	4.1	42.1
Run 2	4.7	0.0	1.8	8.3	25.1	4.3	44.2
						Avg	43.1
						r (calc)	2.1
						r (IP585)	7.5

40 mg/kg Jet fuel spike - Agilent WorkBench prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	4.8	0.0	1.8	8.3	25.4	4.2	41.4
Run 2	4.3	0.0	1.7	7.9	24.0	4.1	39.1
						Avg	40.2
						r (calc)	2.3
						r (IP585)	7.1

Conclusion

The Agilent WorkBench was shown to successfully automate the preparation of the calibration standards and samples when measuring FAME in jet fuel using the IP585 GC/MS method. By comparison, it was also shown that good analysis results can be difficult to obtain when using manual preparation techniques that require precise handling very small amounts of samples and reagents. This application note has demonstrated that the WorkBench can achieve better overall method performance compared to manual preparation. Considerable time was saved in avoiding rework and 10 times less reagents used with the WorkBench.

References

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