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

Anthony Macherone and James McCurry, Agilent Technologies, Wilmington DE, USA

Agilent Technologies

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.

As with most instrumental measurements, successful preparation of calibration standards and samples plays a significant role in achieving quality results. For the IP585 method, 1-mL volumes of calibration standards are made using graduated microliter pipettes. Due to the small volumes, these procedures require considerable skill to correctly prepare standards and samples. A better approach would be to automate the preparation using an instrument 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 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.

1. "IP 585/10 "Determination of fatty acid methyl esters (FAME), derived from bio-diesel fuel, in aviation turbine fuel – GC-MS with selective ion monitoring/scan detection method", The Energy Institute, London, UK.

Jet Fuel Contamination with Fatty Acid Methyl Esters (FAMEs)

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

Due to the variety of feedstocks many different saturated and unsaturated FAMEs can be found in biodiesel. Since it would be difficult to measure every possible FAME in jet fuel, the Energy Institute has identified six FAMEs that represent 95% of the potential sources of jet fuel contamination. These six are shown above.

IP 585 Analysis Conditions for the Agilent 5975C GC/MS

GC Conditions	
Inlet	260 ºC, Splitless mode
Column	HP-INNOWAX, 50m x 0.2 mm ID x 0.4 um film (part # 19091N-205)
Column Flow	Helium at 0.6 mL/min. constant flow
Oven Program	150 ºC 5 min,, 12 ºC /min. to 200 ºC for 17 min., 3 ºC/min. to 252 ºC for 6.5 min
Mass Spec Conditions	
Ionization Source	70 eV electron ionization
Scan Range	33 to 320 AMU
SIM Ions	See table SIM Table.

Detected FAME	SIM Ions	SIM Group Start Time
C16:0	227, 239, 270,271	20.0 min.
C17:0	317	28.0 min.
C17:0-d33 (Int. Std.)	241, 253, 284	28.0 min.
C18:0	255, 267, 298	34.0 min.
C18:1	264, 265, 296	36.5 min.
C18:2	262, 263, 264, 294, 295	38.0 min.
C18:3	236, 263, 292, 293	40.0 min.

WorkBench Preparation of Calibration Standards

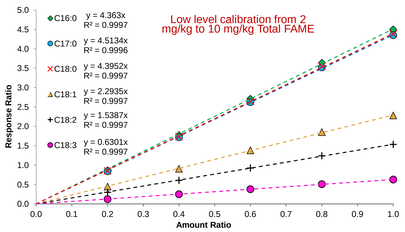
The IP585 method uses ten working calibration standards (WCS) to calibrate the GC/MS system. The WCS are prepared from a Working Standard Solution (WSS) containing 1000 mg/kg of each FAME in n-dodecane. The linear dilution scheme shown below is described in the method to manually prepare 1 mL quantities of each WCS.

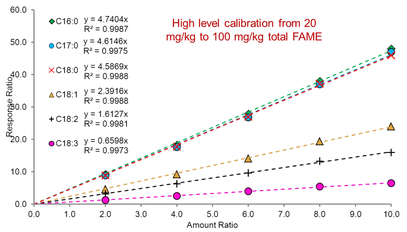
Volume (µL) of Working Standard Solution (WSS)	Volume (µL) of n-C_{12} Solvent	Volume (µL) of Internal Standard (ISTD)	Final Concentration (mg/kg) of Each FAME
1000	0	10	100
800	200	10	80
600	400	10	60
400	600	10	40
200	800	10	20
100	900	10	10
80	920	10	8
60	940	10	6
40	960	10	4
20	980	10	2
0	1000	10	0

For the automated WorkBench preparation, this manual scheme shown above was translated to produce 100 µL of each WCS. The translated linear dilution scheme is shown below.

Volume (µL) of Working Standard Solution (WSS)	Volume (µL) of n-C_{12} 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

The method requires the construction of a low calibration (2 to 10 mg/kg) and a high calibration (20 to 100 mg/kg). The individual FAME calibration curves resulting from the low and high level WorkBench prepared standards are shown below. 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 below. For the low level calibrations, the slopes of the manual and WorkBench calibrations are very similar and the R^2 values 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. The WorkBench prepared standards easily met the method requirements, while the manually prepared standards failed the linearity tests. In this case, the manually prepared samples could not be run until the high level standards were re-made and the calibrations re-verified. This adding considerable time in obtaining results for the manually prepared samples. However, since the WorkBench calibrations were initially correct, the WorkBench samples could be run immediately.

Low Level Calibration
Comparison of Manual and WorkBench Standards

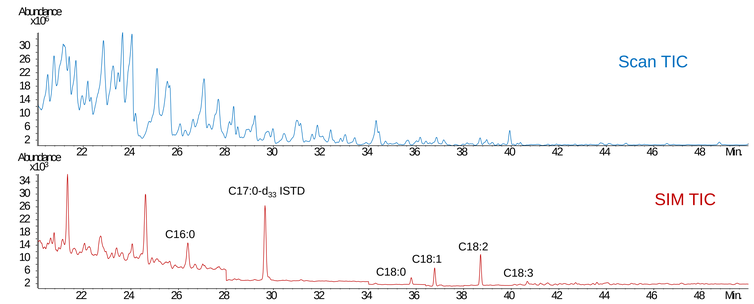
	Slope		R^2	
FAME	Manual	WorkBench	Manual	WorkBench
C16:0	2.941	2.941	1.000	0.999
C17:0	2.441	2.544	1.000	1.000
C18:0	2.664	2.684	1.000	0.999
C18:1	1.539	1.545	1.000	0.999
C18:2	1.105	1.090	1.000	0.999
C18:3	0.478	0.475	1.000	0.999

High Level Calibration
Comparison of Manual and WorkBench Standards

	Slope		R^2	
FAME	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

WorkBench Preparation of Jet Fuel Samples

For the IP585 method, samples are prepared manually by pipetting 1 mL of jet fuel into a 2-mL vial followed by the addition of 10 µL of the internal standard solution. Automated sample preparation was done by having the WorkBench pipet 100 µL of jet fuel into an empty 2-mL vial follow by addition of 1 µL of the internal standard solution. Three jet fuels samples containing different levels of FAME contamination were prepared in duplicate using both the manual procedure and the WorkBench. Each sample duplicate was run on the Agilent 5975C GC/MS system configured for the IP 585 method. Shown below is a typical SIM/SCAN chromatogram of a WorkBench prepared jet fuel sample containing 6 mg/kg of total FAME contamination.



Comparisons of the analysis results for the manually prepared and the WorkBench prepared jet fuels are shown in below. 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 single user precision measurement 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 jet fuel sample #2 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 calculated repeatability were much better than the method's specifications.

Jet Fuel #1 - Manual Sample Prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run1	0.8	0.0	0.1	0.3	0.1	0.0	1.3
Run2	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

Jet Fuel #1 - WorkBench Sample Prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run1	0.8	0.0	0.1	0.3	0.1	0.0	1.3
Run2	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

Jet Fuel #2 - Manual Sample Prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run1	1.9	0.0	0.4	1.7	3.9	1.2	9.1
Run2	1.3	0.0	0.3	1.2	2.7	0.7	6.2
						Avg	7.7
						r (calc)	2.9
						r (IP585)	1.7

Manual prep fails method precision

Jet Fuel #2 - WorkBench Sample Prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run1	1.3	0.0	0.2	1.2	2.8	0.5	6.0
Run2	1.3	0.0	0.3	1.2	2.8	0.6	6.2
						Avg	6.1
						r (calc)	0.2
						r (IP585)	1.5

Jet Fuel #3 - Manual Sample Prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run1	5.2	1.8	8.2	24.1	4.1	0.0	43.4
Run2	5.5	0.0	1.9	8.6	25.2	4.3	45.4
						Avg	44.4
						r (calc)	2.0
						r (IP585)	7.7

Jet Fuel #3 - WorkBench Sample Prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run1	5.2	0.0	1.8	8.2	23.9	4.0	43.1
Run2	5.2	0.0	1.8	8.2	23.8	4.0	43.0
						Avg	43.1
						r (calc)	0.1
						r (IP585)	7.5

Summary and Conclusion

The Agilent 7696A WorkBench completely automates standard and sample prep for the analysis of trace FAMEs in Jet Fuel using IP method 585

- Uses 10x less chemical resources
  - 100 uL WorkBench prep vs. 1 mL manual prep
  - Saves expensive C17:0-d_{33} internal standard solution
- Improves Calibration Performance
  - Better high level calibration linearity compared to manually prepared standards
- Improves Sample Precision
  - Better repeatability compared to manually prepared samples
  - Fewer sample prep errors
  - Consistent long-term precision