



# Automated Standard and Sample Preparation for Multiple Gas Chromatographic Analyses of Biodiesel

## Application Note

HPI/Petrochemical/Polymer

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### Abstract

An approach to automating the biodiesel standard and sample preparation prior to GC analysis is presented using the enhanced capabilities of the Agilent 7693A Automated Liquid Sampler (ALS). A derivatization protocol for the silylation of glycerol, mono-, and di-glycerides was developed for the 7693A ALS using a procedure described in the ASTM D6584 and EN14105 methods [1,2]. A second automated protocol was developed for adding an internal standard to biodiesel samples prior to the fatty acid methyl ester (FAME) analysis according to the EN14103 method [3]. The resulting calibrations and GC sample runs of these methods shows excellent comparison to manual preparation methods.



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## Introduction

Several industry standard gas chromatographic methods are used to determine the product quality of pure biodiesel, also known as B100. Two of these methods, ASTM D6584 and EN14105, require very complex and time consuming procedures for preparing calibration standards and finished B100 samples. These methods also use expensive and toxic reagents adding to the cost of analysis. While modern instruments possess many automation capabilities with regards to the gas chromatographic process, manual sample and standard preparation remains a tedious chore that can significantly contribute to errors and lost precision in GC analyses.

The new Agilent 7693A ALS offers new capabilities for automatic standard and sample preparation at the GC instrument. This paper describes these capabilities for improving the productivity of three biodiesel GC methods. Both ASTM D6584 and EN14105 require the derivatization of non-volatile glycerides in B100 prior to GC analyses. Additionally, the calibration standards for these methods must also undergo the same derivatization procedure. Careful configuration of the Agilent 7890A GC with the 7693A ALS can eliminate most manual preparation steps for the D6584 and EN14105 methods. A third biodiesel method, EN14103, is used to measure the fatty acid methyl ester (FAME) and linolenic acid methyl ester content in B100 samples. This method requires the addition of an internal standard solution to each B100 sample. This sample preparation step can also be performed using the 7693A ALS.

## Experimental

An Agilent 7890A GC was configured according to Table 1. In addition to this configuration, an optional isothermal, external column oven was placed on the top of the GC to accommodate an HP-INNOWax column for the EN14103 analysis. This was necessary to avoid damage to this column when the high temperature D6584 or EN14105 methods were run. GC conditions for these methods are listed in Tables 2 and 3. This hardware configuration allows up to five different GC methods for biodiesel analysis to be run on a single instrument:

- **ASTM D6584** - Determination of Free and Total Glycerin and Mono-, Di-, Triglyceride Content
- **EN14105** - Determination of Free and Total Glycerol and Mono-, Di-, Triglyceride Content
- **EN14103** - Determination of Ester and Linolenic Acid Methyl Ester Content
- **EN14110** - Determination of Methanol Content
- **EN14106** - Determination of Free Glycerol

Table 1. 7890A GC Configuration for 5-in-1 Biodiesel Analyses

Front inlet	Split/splitless with split optimized liner (5188-6576)
Rear inlet	Cool-on-column
Column 1	HP-INNOWax, 30 m × 0.32 mm id × 0.25 μm film (19091N-113V) - special configuration for external column oven
Column 2	DB-5ht, 15 m × 0.32 mm id × 0.1 μm film (123-BD11) with 2 m × 0.53 mm id retention gap (160-2865-5)
Detectors	Dual flame ionization (FID)
Firmware version	A.01.10 or greater
Data system	Chemstation rev. B.04.01 or greater

Table 2. GC Conditions for Analysis of Free and Total Glycerin in B100 Biodiesel (ASTM D6584 or EN14105)

Cool-on-column inlet	
Initial temperature	50 deg °C
Temperature program	Oven track mode
Column 2 (DB-5ht) flow	Helium at 3 mL/min measured at 50 deg °C
Column temperature	
Initial	50 °C for 1 min
Rate 1	15 °C/min to 180 °C, hold 0 min
Rate 2	7 °C/min to 230 °C, hold 0 min
Rate 3	30 °C/min to 380 °C, hold 10 min
Flame ionization detector	380 °C

Table 3. GC Conditions for Determination of Ester and Linoleic Acid Methyl Ester Content (EN14103)

Split/splitless inlet	
Temperature	250 °C
Split flow	100 mL/min
Column 2 (HP-INNOWax) flow	Helium at 1 mL/min
Column temperature	210 °C for 30 min.
Flame ionization detector	250 °C

Combining automated sample preparation and automated sample injection with GC analysis was accomplished using two 7693A towers and the 7693A sample tray. One tower was used for sample preparation while the second tower was used to inject the prepared sample onto the GC column. Special accessories on the sample tray were used to mix and heat samples as required by the preparation protocols. Table 4 lists the individual 7693A ALS components used for this paper. Control of the towers and sample tray was accomplished using control software built into the Agilent Chromatography Data systems.

Table 4 - 7693A ALS Configuration

Front tower	7693A Autoinjector (G4513A)
Rear tower	7693A Autoinjector (G4513A)
Sample tray	7693A with heater, mixed, bar code reader (G4520A)
Sample preparation syringe	100 μL, PTFE plunger (5183-2042)
GC Inlet injection syringe	10 μL PTFE plunger (5181-3354)

Table 5 lists the standards and reagents necessary for ASTM D6584 and EN14105. The preparation procedures described by these methods result in a final volume of 15 mL for each sample. The maximum vial size used by the 7693A ALS is 2 mL. Therefore the standard and sample preparation procedures must be scaled to 10% for the final volume to fit into the 2 mL vials. Shown below is the 10% scaled protocol used by the 7693A ALS for the D6584 standard preparation. The same protocol was used for the EN14105 standard preparation.

1. Move an empty 2 mL vial from the sample tray to the front tower.
2. Add 100  $\mu$ L calibration standard mixture #1 to vial (100  $\mu$ L syringe).
3. Add 10  $\mu$ L ISTD1 solution (butanetriol) to vial using front tower.
4. Add 10  $\mu$ L ISTD2 solution (tricaprin) to vial using front tower.
5. Add 10  $\mu$ L derivatization reagent (MSTFA) to vial using front tower.
6. Transfer vial to mixer and mix for 1 minute.
7. Transfer vial to heater and react for 30 minutes at room temperature.
8. Transfer vial to front tower.
9. Add 800  $\mu$ L n-heptane to vial using front tower.
10. Transfer vial to mixer and mix for 1 minute.
11. Transfer vial to rear tower (10  $\mu$ L syringe).
12. Inject 1  $\mu$ L on-column using rear tower.
13. Repeat for calibration standard mixes, numbers 2 - 5.

Table 5. Standards and Reagents used for Analysis of Free and Total Glycerin in B100 Biodiesel

ASTM D6584 standard kit (5190-1408)	Contains 2 pre-mixed internal standards and 5 pre-mixed calibration standards
EN14105 standard kit (5190-1409)	Contains 2 pre-mixed internal standards and 4 pre-mixed calibration standards
MSTFA derivatization reagent (5190-1407)	Contains 10 $\times$ 1 mL ampoules of N-methyl-N-(trimethylsilyl) trifluoro-acetamide
Monoglyceride retention time standard (5190-1410)	Contains 3 $\times$ 1 mL ampoules of pre-mixed monoglycerides for retention time identification

The B100 sample preparation protocol was slightly different. First, it was necessary to weigh 10 mg of each soybean B100 sample into separate 2 mL ALS vials. After the samples were weighed into the vials, they were placed in the 7693A sample tray for automated preparation using this protocol:

1. Move vial containing 10 mg B100 sample from sample tray to front tower.
2. Add 10  $\mu$ L ISTD1 solution (butanetriol) to vial using front tower.
3. Add 10  $\mu$ L ISTD2 solution (tricaprin) to vial using front tower.
4. Add 10  $\mu$ L derivatization reagent (MSTFA) to vial using front tower.
5. Transfer to vial mixer and mix for 1 minute.
6. Transfer to vial heater and react for 30 minutes at room temperature.
7. Transfer vial to front tower.
8. Add 800  $\mu$ L n-heptane to vial using front tower.
9. Transfer vial to mixer and mix for 1 minute.
10. Transfer vial to rear tower (10  $\mu$ L syringe).
11. Inject 1  $\mu$ L on-column using rear tower.
12. Repeat for other B100 samples.

The sample preparation required by EN14103 is much simpler. Three B100 samples sourced from rape seed oil, palm oil, and coconut oil were used for this study. After 10 mg of each B100 sample was weighed into 2 mL ALS vials, the following 7693A protocol was used for sample preparation:

1. Move vial containing 10 mg B100 sample from sample tray to rear tower (100  $\mu$ L syringe).
2. Add 500  $\mu$ L ISTD Solution (10 mg/mL C17:0 in n-heptane) to vial using rear tower (100  $\mu$ L Syringe).
3. Transfer vial to mixer and mix for 1 minute.
4. Transfer vial to front tower (10  $\mu$ L syringe).
5. Inject 1  $\mu$ L onto split inlet using front tower (10  $\mu$ L syringe).
6. Repeat for other B100 Samples.

## Results

### ASTM D6584 - Determination of Free and Total Glycerin and Mono-, Di-, Triglyceride Content

The automated standard preparation resulted in a 5-level calibration for glycerol, monoolein, diolein and triolein with two internal standards. Figure 1 shows the chromatograms generated for each standard. Visually, it was difficult to determine if the standards were prepared correctly using the 7693A ALS. A better representation of properly prepared standards was the individual linear calibration curves shown in Figure 2. This data shows excellent linearity for each compound that exceeds the requirements of ASTM D6584 ( $r^2 = 0.99$  or

greater). Figure 3 shows a comparison of a soybean B100 sample prepared manually and automatically. Details of the four different glyceride elution regions are shown in Figure 4. This data shows that the 10% scaled automated sample preparation yields the same chromatographic result as the full manual sample preparation.

### EN14103 - Determination of Ester and Linolenic Acid Methyl Ester Content

Figures 5, 6 and 7 show three different biodiesel samples analyzed using the EN14103 method. For each sample, the automated sample preparation using the 7693A ALS yielded the same chromatographic result as the manual preparation.

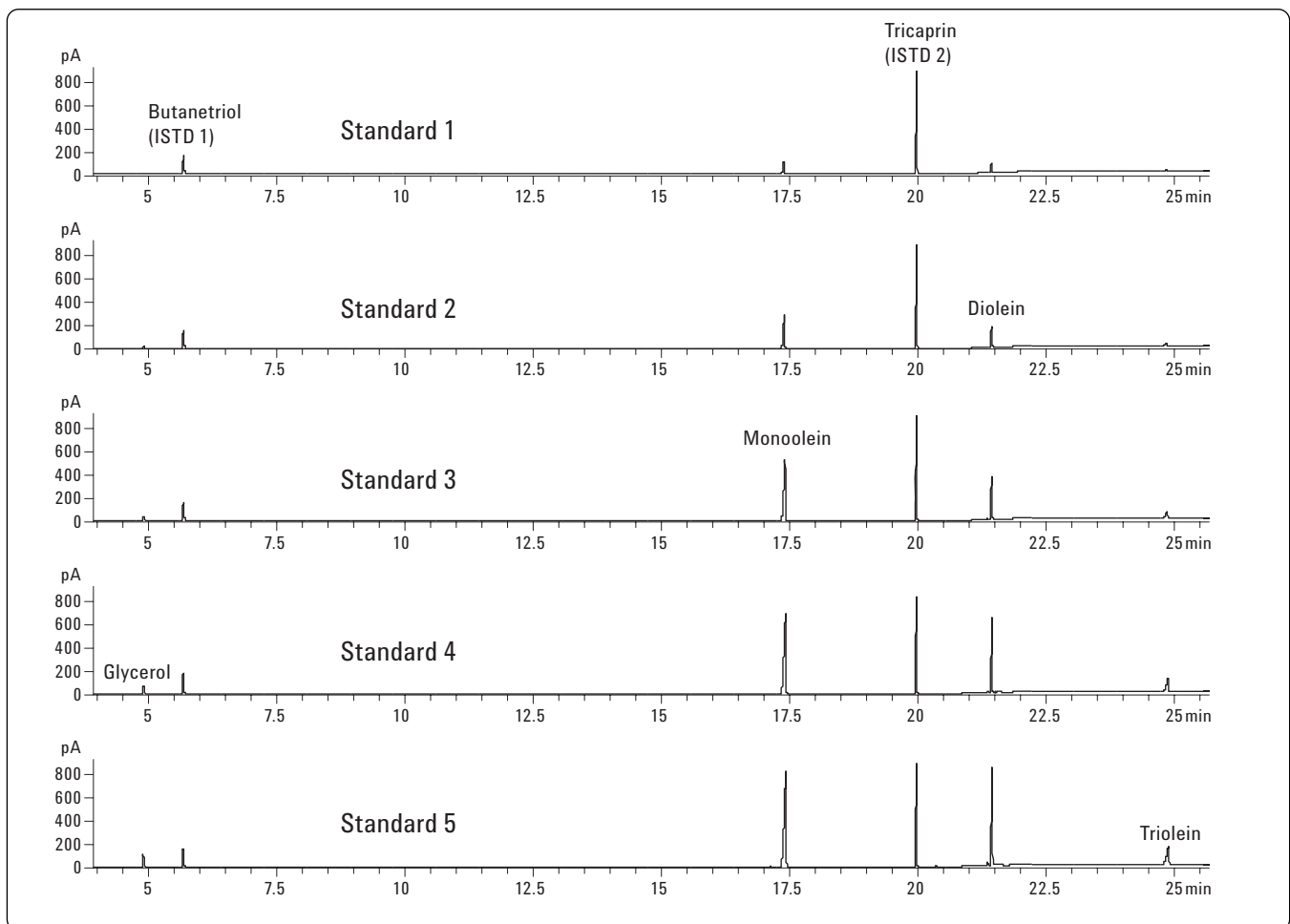


Figure 1. A five level ASTM D6584 calibration was automatically prepared using the Agilent 7693A ALS and run on the Agilent 7890A Biodiesel Solution.

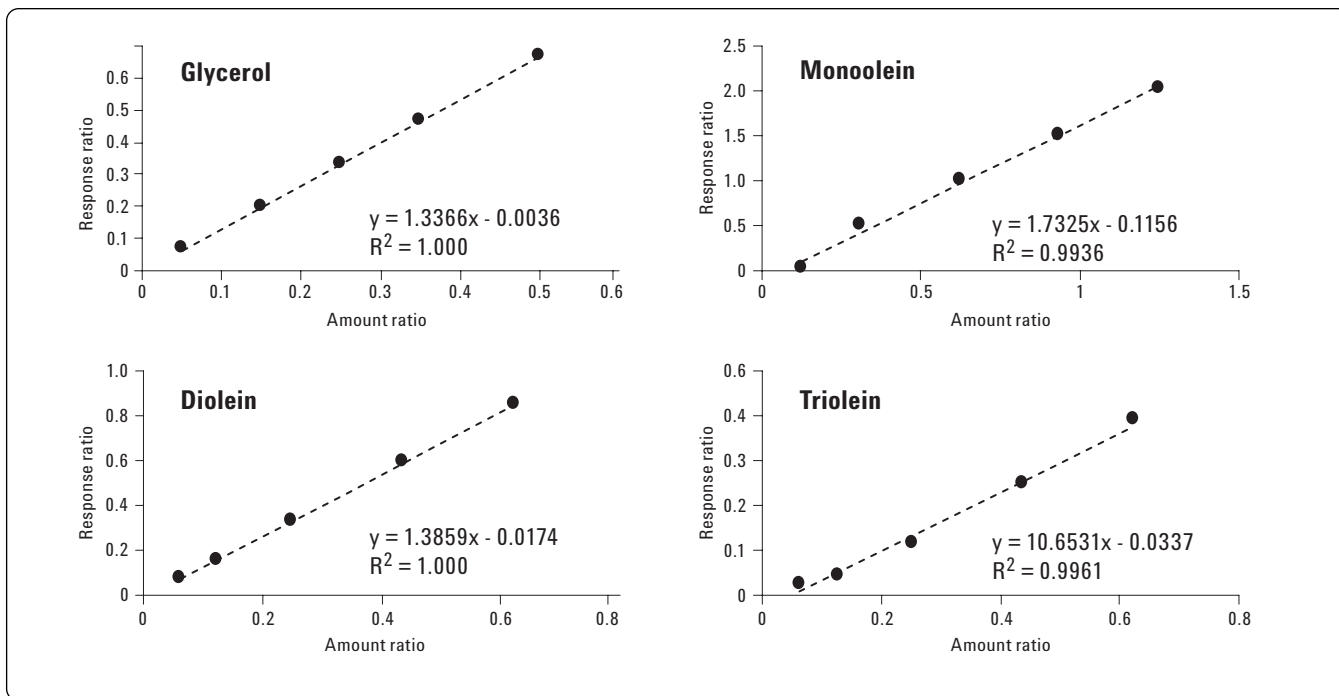


Figure 2. Five point linear calibration curves resulting from automated standard preparation with the Agilent 7693A ALS.

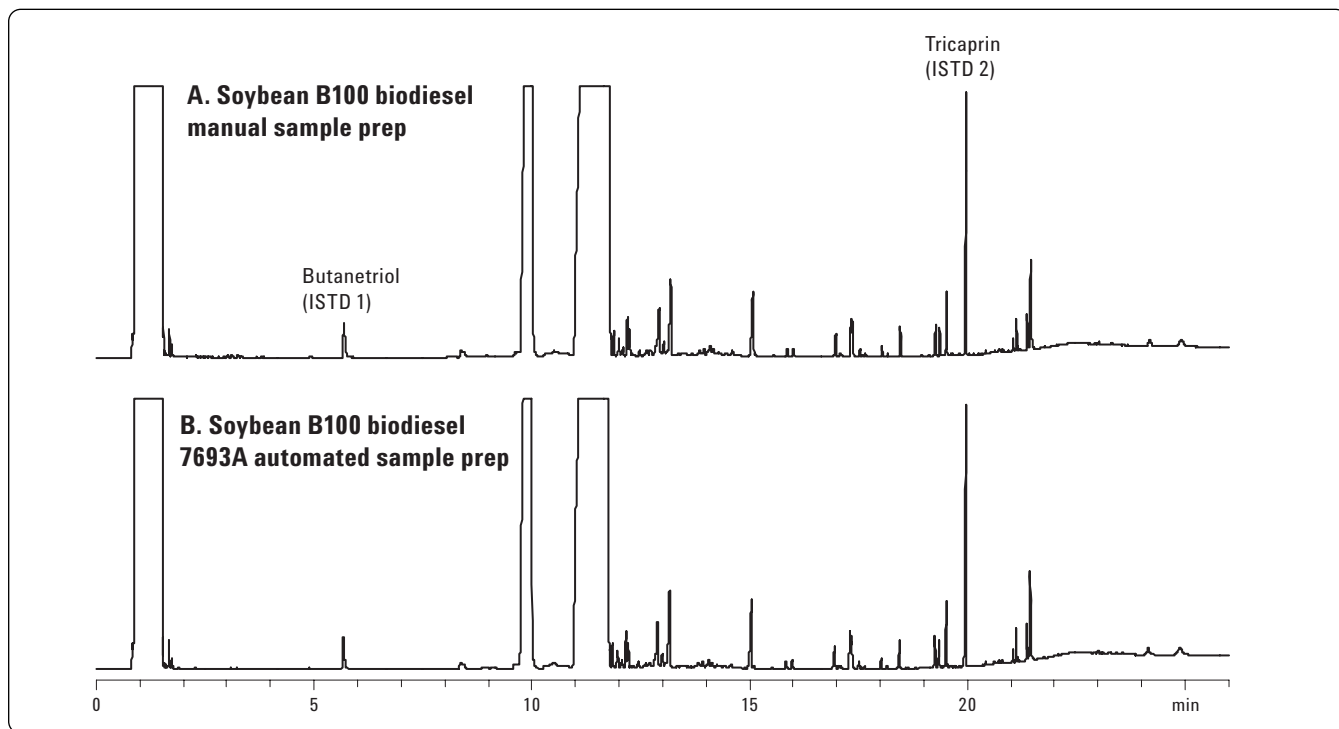


Figure 3. Comparison of a soybean B100 sample prepared (A) manually according to the ASTM D6584 protocol and (B) the automatic 10% scaled protocol using the Agilent 7693A ALS and Agilent 7890A Biodiesel Solution.

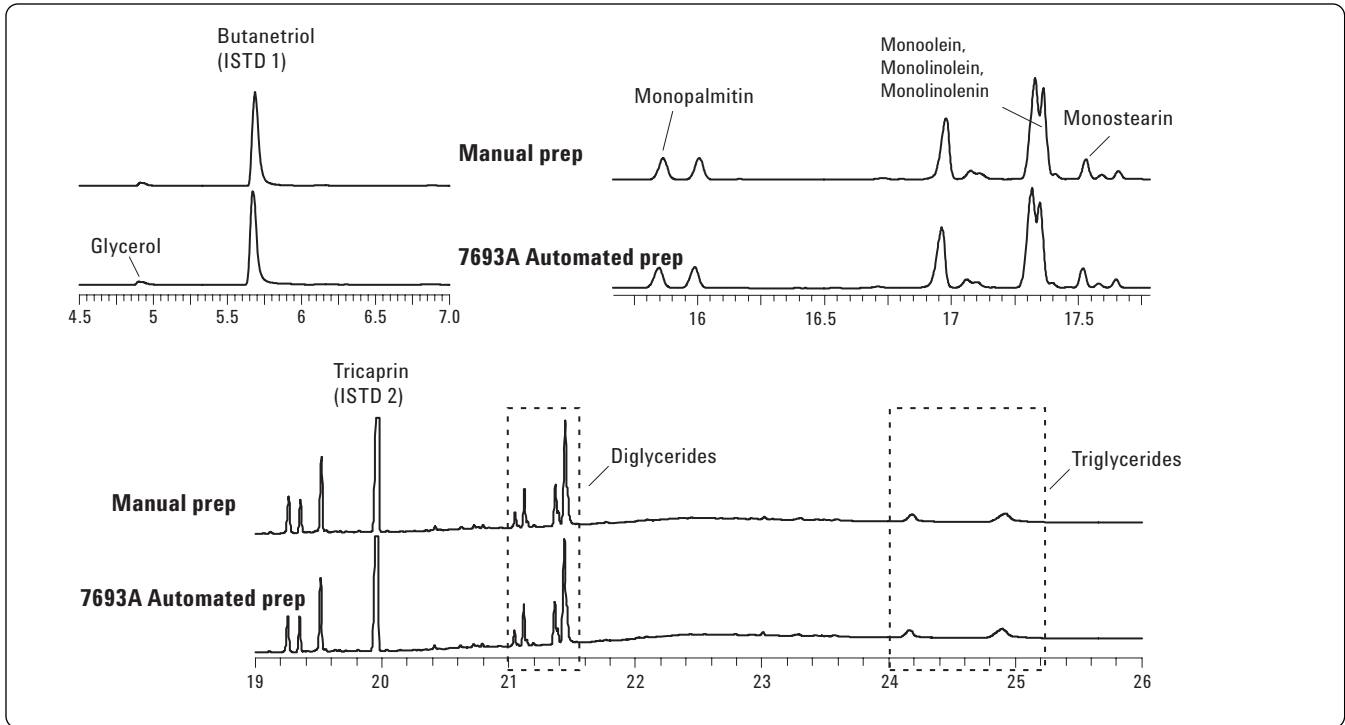


Figure 4. Details of the glycerol, mono-, di-, and tri-glycerides contained in a soybean B100 sample. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

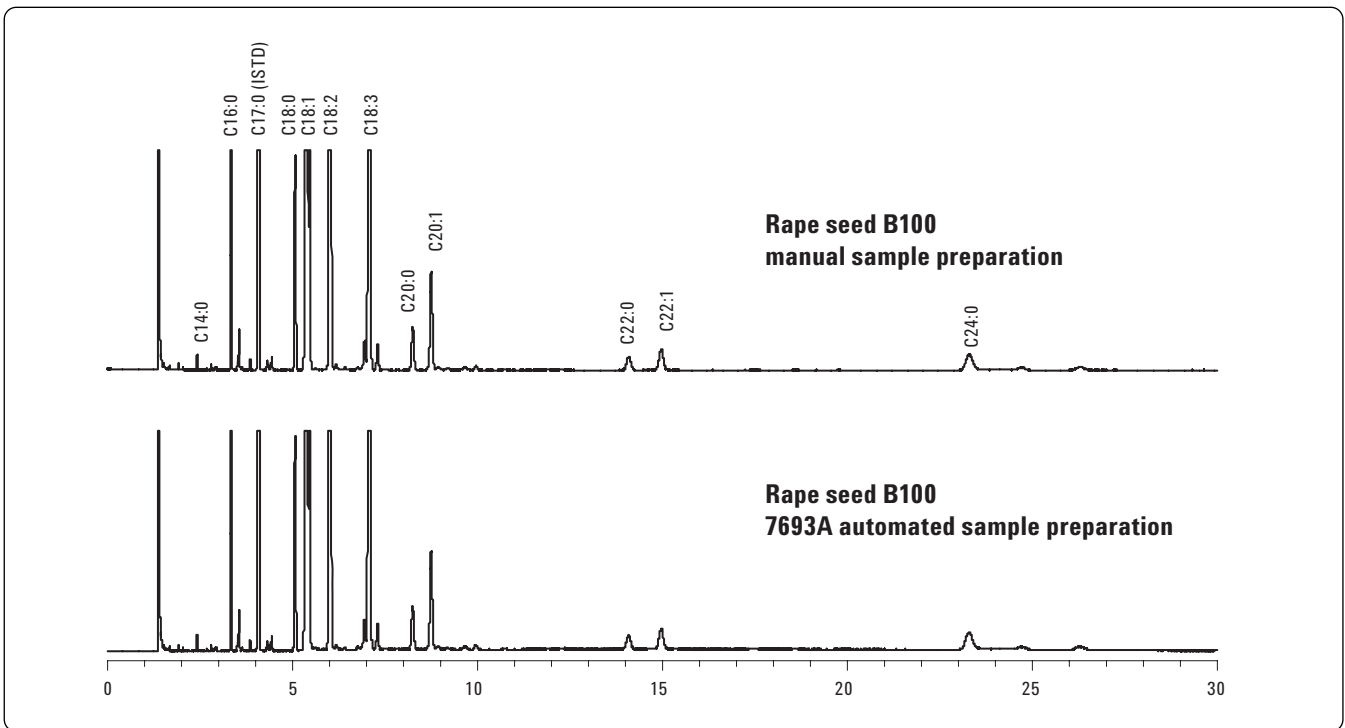


Figure 5. Analysis FAMES in rape seed B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

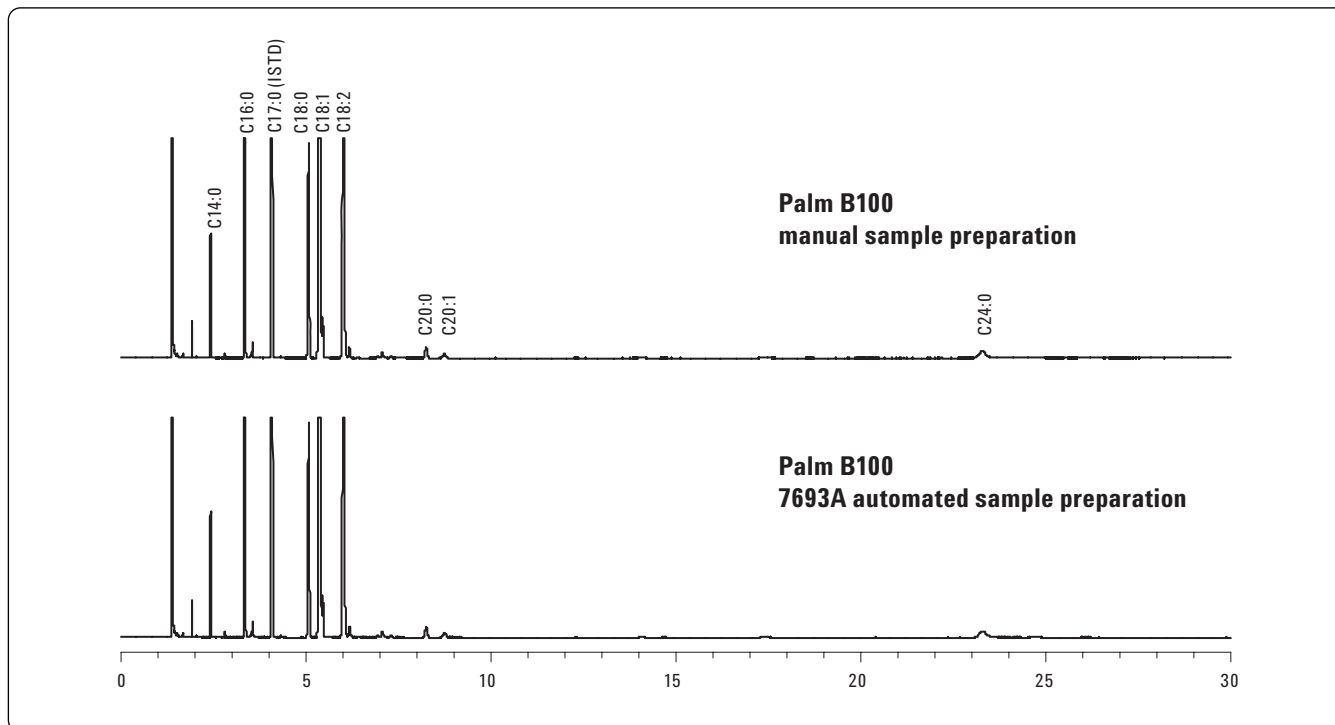


Figure 6. Analysis FAMES in palm oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

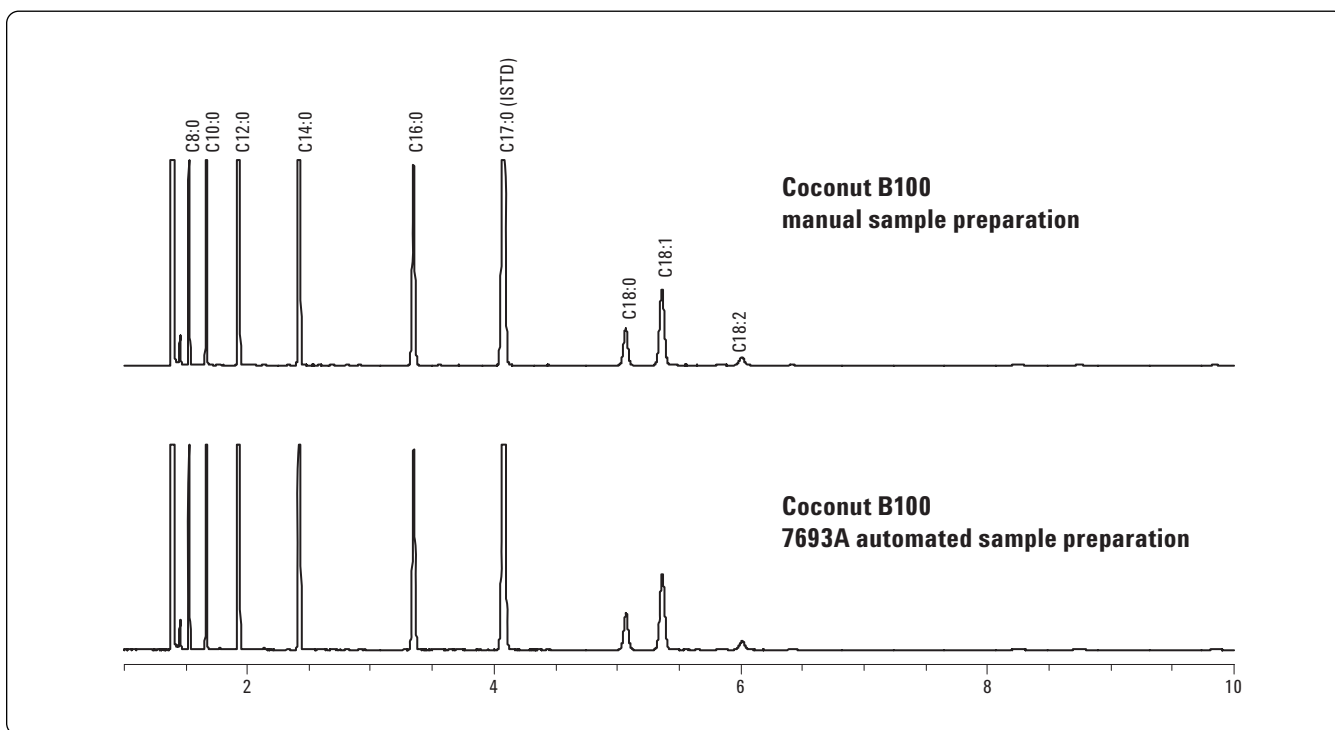


Figure 7. Analysis FAMES in coconut oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

## Conclusions

In the past, measuring biodiesel quality was divided into two parts: sample preparation and GC analysis. Sample preparation was complex, becoming a major contributor to errors and the need to repeat sample analyses. This paper demonstrates the automated sample and standard preparation capabilities of the Agilent 7693A ALS applied to the analysis of B100 biodiesel. A complex manual derivatization procedure used by the ASTM D6584 and EN14105 methods for measuring free and total glycerine was successfully converted to a completely automated protocol with the 7693A ALS. This sample preparation protocol was integrated with the GC analysis conditions on the Agilent 7890A Biodiesel Analysis Solution creating a totally automated system. An automated sample preparation and GC analysis was also developed for the analysis of FAMES and methyl linolenate using method EN14103. Automated sample preparation combined with automated GC analysis offers improved reliability and productivity for laboratories concerned with obtaining the highest quality results for biodiesel analyses. Additionally, automated sample preparation can reduce the usage of expensive reagents and standards and the exposure of laboratory personnel to toxic materials.

## References

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