Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

9. Calibration and Standardization

9.2 *Standard Solutions* – Prepare the five standard solutions in ***Table 3*** by transferring the specified volumes by means of Microliter syringes to 10mL septa vials. Add to each of the five standard solutions 100uL of MSTFA. Close the vial and shake. Allow the vial to stand for 15 to 20 minutes at room temperature. Add approximately 8 mL n-Heptane to vial and shake.

TABLE 3 Standard Solutions					
Standard Solution Number	**1**	**2**	**3**	**4**	**5**
uL of glycerin stock solution	10	30	50	70	100
uL of monoolein stock solution	20	50	100	150	200
uL of diolein stock solution	10	20	40	70	100
uL of triolein stock solution	10	20	40	70	100
uL of butanetriol stock solution	100	100	100	100	100
uL of tricaprin stock solution	100	100	100	100	100

9.4 *Standardization*

9.5 Inject 1 uL of the reaction mixture into the cool-on-column injection port and start the analysis.

_____

10. *Procedure*

10.1 Weigh to the nearest 0.1 mg approximately 100 mg of sample directly into a 10 mL septa vial. Using Microliter syringes, add exactly 100 uL of each internal standard and MSTFA. Shake the vials, and allow to set for 15 to 20 minutes at room temperature. Add approximately 8 mL of n-Heptane to vial and shake.

10.2 Inject 1 uL of the reaction mixture into the cool on-column injection port and start the analysis.

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

System shown with Agilent 7890 and Twin PAL.
The Lower PAL is the Prep PAL and runs on Cycle Composer Software. All of the sample prep is performed with this PAL. There is a 100ul syringe for the solvent transfers. There are (7) 2mL solvent locations. There is a DISPTool that is hooked up to a 5 mL dilutor syringe for the 8 mL Heptane addition. There is a fast wash station that Wash1 is filled with Toluene and Wash 2 is filled with Heptane. There is an agitator that is set at 35 deg C and 500RPM to do the mixing.

The Upper PAL is the Inject PAL and runs under ChemStation Software control. This requires Agilent CTC Control Software supplied by Agilent. It is using a 5 ul syringe with a tapered needle to do 1 ul on column injections into the rear injection port. There is a fast wash station that Wash1 is filled with Toluene and Wash 2 is filled with Heptane.

The expected **Sample Prep Time** is approximately **26 minutes**
The expected **GC Runtime** is approximately **31.81 minutes**
The expected **GC Cool down time** is approximately **7 minutes**

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

10. *Cycle Composer* **LEAP Method – D6584 Sample Prep w IS MSTFA Rev3**
**10.1** Weigh to the nearest 0.1 mg approximately 100 mg of sample directly into a 10 mL septa vial. Using Microliter syringes, add exactly 100 uL of each internal standard and MSTFA. Shake the vials, and allow to set for 15 to 20 minutes at room temperature. Add approximately 8 mL of n-Heptane to vial and shake.
LEAP Hardware - Using a 100ul Syringe add IS#1 from ReagentReservoir#5, add IS#2 from ReagentReservoir#6, Add 100ul of MSTFA from ReagentReservoir#7. Take to Agi and shake. Allow sample to equilibrate for 15 minutes. Add 8 mL of n-Heptane with diluter hooked to DISPTool. Take back to Agi and shake. Take back to home location.
(Only one sample is processed at a time as there is a time limit once adding the MSTFA)
The expected time of each sample prep is 26 minutes. When injecting with the Inj PAL there is an overlap that the 2nd sample on is prepped while the GC is running.

Reservoir #	Stock Solution	uL
1	glycerin stock solution	0
2	Monoolein stock solution	0
3	diolein stock solution	0
4	triolein stock solution	0
5	butanetriol stock solution (Internal Standard #1)	100
6	tricaprin stock solution (Internal Standard #2)	100
7	MSTFA	100
DISPTool	n-Heptane	8000
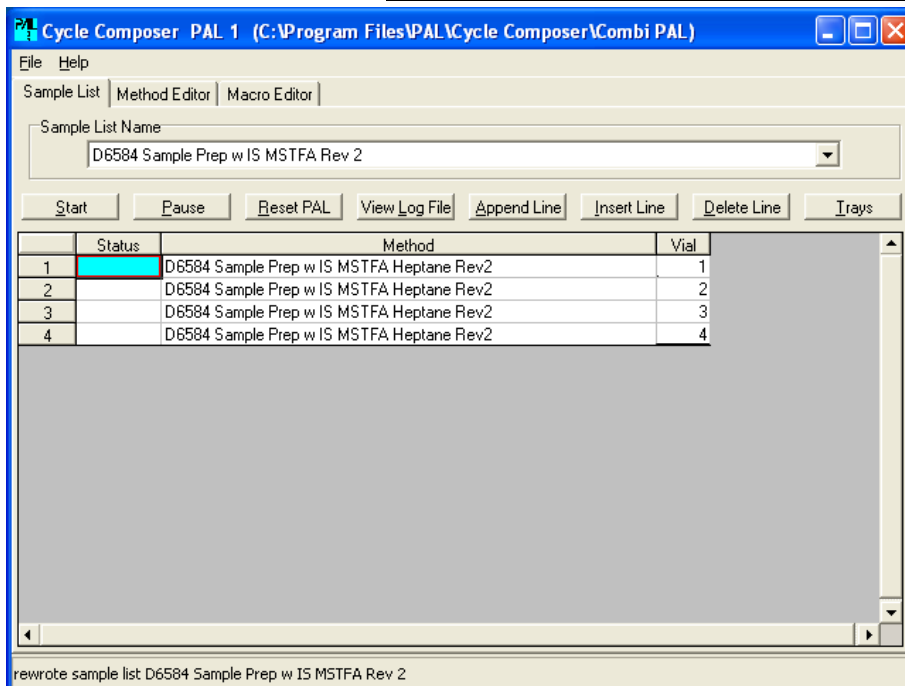
Cycle Composer
Choose the sample list **D6584 Sample Prep w IS MSTFA Rev3**

It is important to add or remove samples in the list for the number that are being run.

Note: Cycle Composer and ChemStation need to match on the number of vials that are going to be run

Cycle Composer needs to start before ChemStation method to set appropriate sync signals

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

ChemStation **LEAP Method – LEAPInjection6584**
10.2 Inject 1 uL of the reaction mixture into the cool on-column injection port and start the analysis.
LEAP Hardware - Using a 5ul Syringe pull 1 uL of sample and inject into rear cool-on column injection port and start GC. *(Note: Ensure the proper tapered needle is installed to fit into the injector)*

Pick the ChemStation Sequence named **LEAPINJECTION6584** and it will look like the one below.

The Location field is P1-A-01 for the first vial location. P1-A-02 is the second vial and so on. It is necessary for the format to be followed.

The injector is not chosen from here so it can be ignored.

To begin the sequence chooses the Run Sequence button.
Note: The Cycle Composer sample list number of vials needs to match the ChemStation sequence table vials
Note: Cycle Composer needs to start before ChemStation method to set appropriate sync signals

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

9. Calibration and Standardization

9.2 *Standard Solutions* –

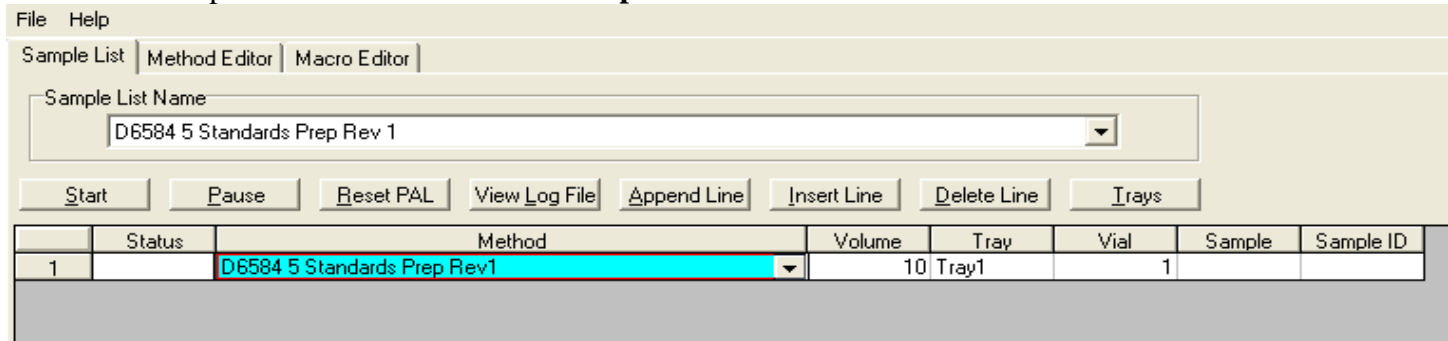**LEAP Method – D6584 5 Standards Prep Rev1**

Prepare the five standard solutions in *Table 3* by transferring the specified volumes by means of Microliter syringes to 10mL septa vials.

LEAP Hardware - Using a 100ul syringe, beginning with glycerin Standard #5 and working back to Standard #1. Beginning with #5 allows us to have the larger volumes dispensed first on a consistent basis. For volumes over 100ul we make multiple trips with the total amount split in half. Wash syringe only when changing stock solutions. The expected time of the 5 standards prep is 22 minutes. Does not use the Inj PAL.

TABLE 3 Standard Solutions					
Standard Solution Number	1	2	3	4	5
uL of glycerin stock solution	10	30	50	70	100
uL of monoolein stock solution	20	50	100	150	200
uL of diolein stock solution	10	20	40	70	100
uL of triolein stock solution	10	20	40	70	100
uL of butanetriol stock solution	100	100	100	100	100
uL of tricaprin stock solution	100	100	100	100	100

Cycle Composer

Choose the sample list **D6584 5 Standards Prep Rev 1**

Note: ChemStation should not be running any methods while this is being run to avoid collisions of the PALs.

Reference Chart for total volumes required for 5 standards to be made

| Reservoir #	Stock Solution	uL	uL	uL	uL	uL	TotalVolume uL
1	glycerin stock solution	10	30	50	70	100	260
2	monoolein stock solution	20	50	100	*150*	*200*	520
3	diolein stock solution	10	20	40	70	100	240
4	triolein stock solution	10	20	40	70	100	240
5	butanetriol stock solution (Internal Standard #1)	100	100	100	100	100	500
6	tricaprin stock solution (Internal Standard #2)	100	100	100	100	100	500
7	MSTFA	100	100	100	100	100	500
DISPTool	n-Heptane	8000	8000	8000	8000	8000	40000

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

9.2 *Standard Solutions* **– continued**
LEAP Method – D6584 Standards Prep w MSTFA Rev3
Add to each of the five standard solutions 100uL of MSTFA. Close the vial and shake. Allow the vial to stand for 15 to 20 minutes at room temperature. Add approximately 8 mL n-Heptane to vial and shake.
LEAP Hardware - Using a 100ul Syringe add MSTFA from ReagentReservoir#7. Take to Agi and shake. Allow sample to equilibrate for 15 minutes. Add 8 mL of n-Heptane with diluter hooked to DISPTool. Take back to Agi and shake. Take back to home location *(from sample list)*. The expected time of each standards prep is 22 minutes. .When injecting with the Inj PAL there is an overlap that the 2nd sample on is prepped while the GC is running. *(Only one sample is processed at a time as there is a time limit once adding the MSTFA)*
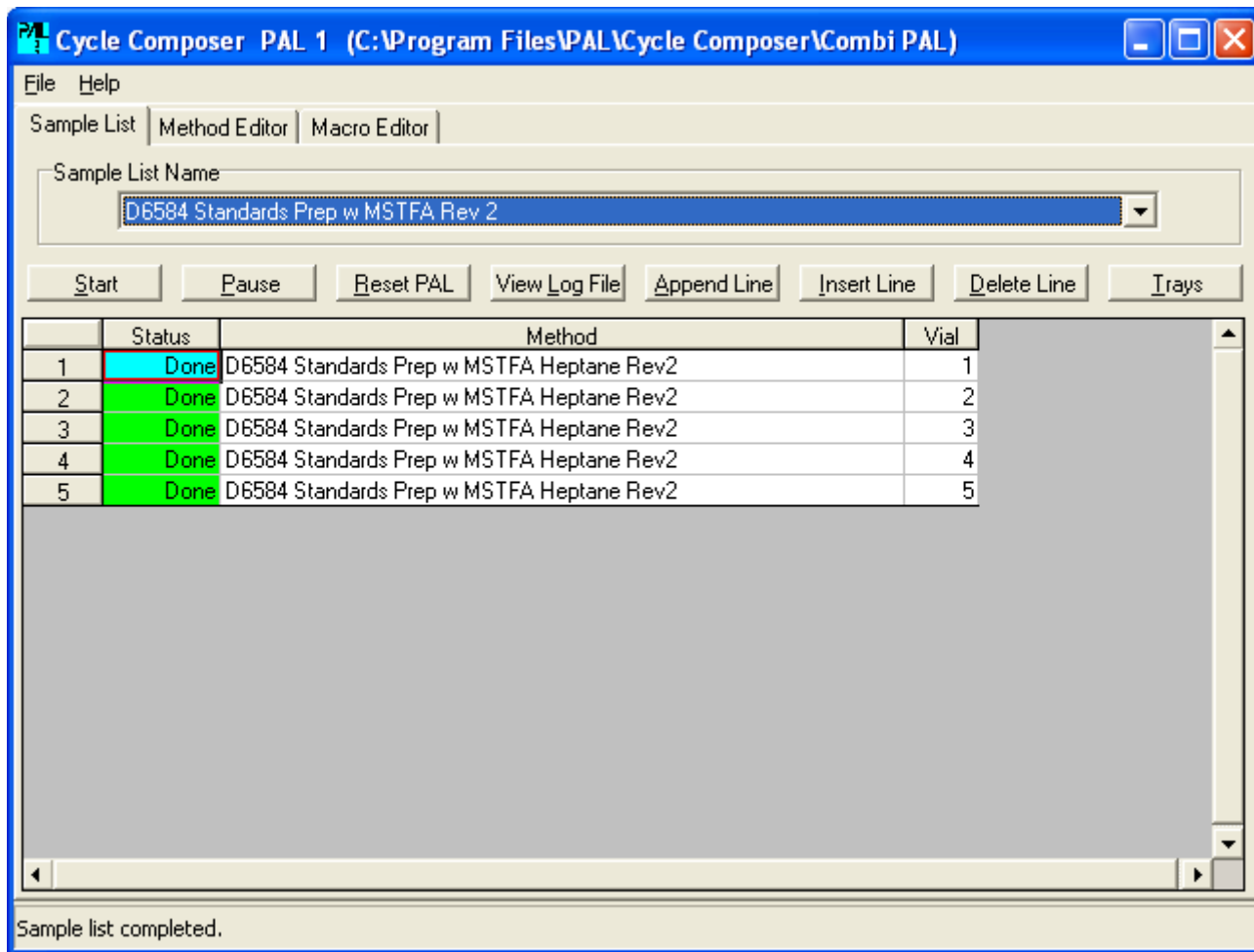
Cycle Composer
Choose the sample list **D6584 Standard Prep w MSTFA Heptane Rev3**

There are 5 lines in the sample list and represent Standard1, Standard2, Standard3, Standard4, Standard5.

Note: The Cycle Composer sample list number of vials needs to match the ChemStation sequence table vials
Note: Cycle Composer needs to start before ChemStation method to set appropriate sync signals

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

9.4 S*tandardization*
ChemStation **LEAP Method – LEAPInjection6584**
9.5 Inject 1 uL of the reaction mixture into the cool-on-column injection port and start the analysis.
LEAP Hardware - Using a 5ul Syringe pull 1 uL of sample and inject into rear cool-on column injection port and start GC. *(Note: Ensure the proper tapered needle is installed to fit into the injector)*

Pick the ChemStation Sequence named **LEAPINJECTION6584** and it will look like the one below

The Location field is P1-A-01 for the first vial location. P1-A-02 is the second vial and so on. It is necessary for the format to be followed.

The injector is not chosen from here so it can be ignored.

To begin the sequence chooses the Run Sequence button.
Note: The Cycle Composer sample list number of vials needs to match the ChemStation sequence table vials
Note: Cycle Composer needs to start before ChemStation method to set appropriate sync signals

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

LEAP Method – Dilutor Prime in Holder Rev1

This method will prime the dilutor 'manually' before a run at its home park position. The PAL Injection Heads do not move.

Note: ChemStation should not be running any methods while this is being run to avoid collisions of the PALs

LEAP Method – Syringe Prime Rev1

This method will prime the Prep syringe 'manually' before a run at its Wash Stations. The Inj PAL is not used.

Note: ChemStation should not be running any methods while this is being run to avoid collisions of the PALs

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

Misc Notes:

Method Editor TAB
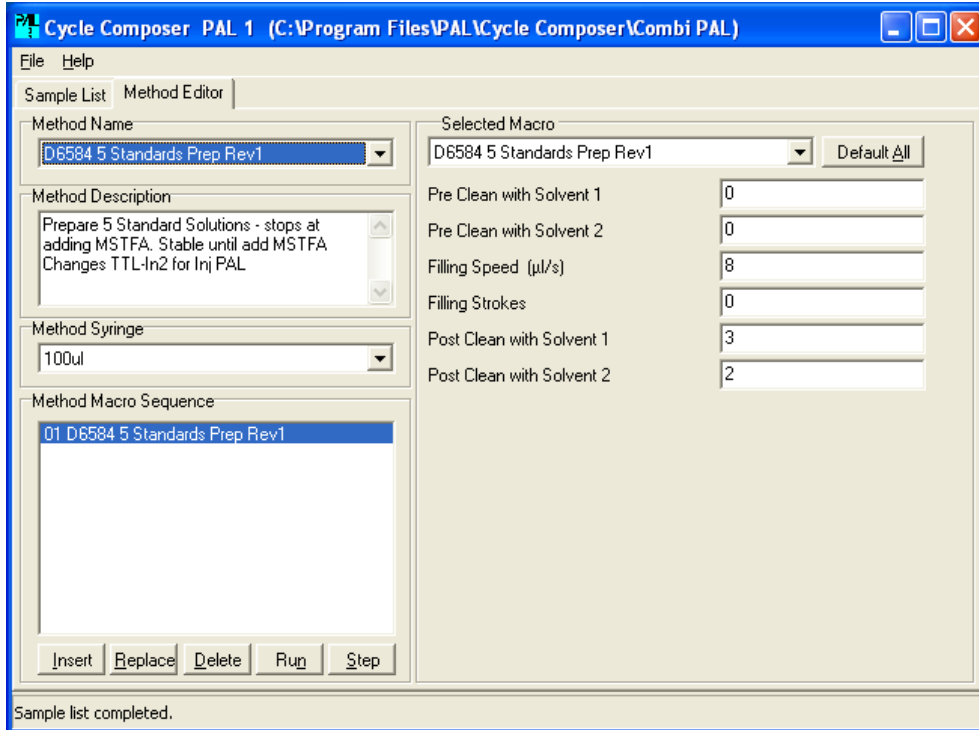
It is possible to change some parameters such as the number of washes with each Solvent.
All of the washes are set at a default of 3 for Wash1 and 2 for Wash2.
Wash 1 = Toluene
Wash2 = Heptane

All of the suggested default parameters are set and can be gotten back to by pressing the Default All button.

Refer to Cycle Composer manual for additional information.

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography
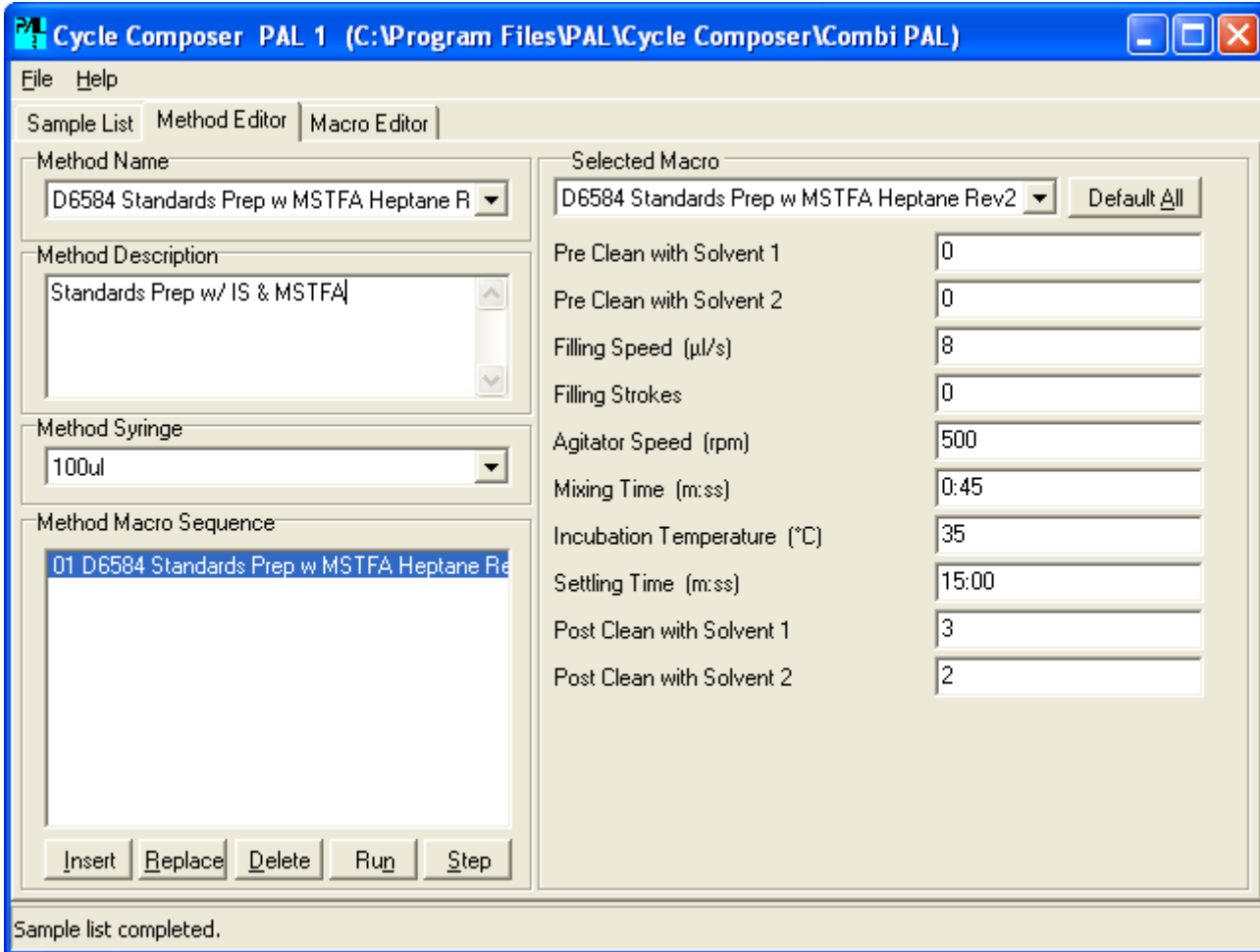
This is representative of the expected macro settings for the Sample and Standards prep based on the ASTM method documentation.

Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography

Start both the CS and CC sequence/sample list

Use the new CS cycle variable "Next Prep Delay" to delay the next sample prep by the CC PAL by 17 minutes.