PROCEDURE	PROCEDURE NUMBER
Quantification of Residual Lactide in Polylactide (PLA) by Gas Chromatography (GC) Using a Flame Ionization Detector (FID)-External Release Version	PLA_GC_13_4
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PRINCIPLE

Lactide polymerization is a reversible reaction. The concentration of lactide in polylactide (PLA) at equilibrium is ~3 wt% at 180 °C. Devolatilization of PLA is capable of lowering residual lactide monomer concentration to <0.3 wt%.

Samples are prepared for residual lactide analysis by adding methylene chloride to a scintillation vial containing PLA sample. Both PLA and lactide are very soluble in methylene chloride solvent. 2,6-dimethyl-□-pyrone internal standard is added to this solution and an aliquot of the resulting solution is transferred to a second scintillation vial containing acetone. Excess cyclohexane is then added to scintillation vial to precipitate PLA, yet retain the residual lactide monomer and internal standard in solution. Supernate solution is then filtered and injected into a gas chromatograph (GC) where the lactide stereoisomers are separated and detected by a flame ionization detector (FID). It is important that GC injector temperature of 200 °C is used to eliminate/minimize any contributions from reformed lactide originating from low MW lactic acid oligomers present in supernate.

SCOPE

This internal standard GC/FID method has been validated to quantify 0.1-5 wt% residual lactide in PLA.

EQUIPMENT AND REAGENTS

Instrumentation/Equipment

1. Agilent 6890 gas chromatograph with split/splitless injector set in splitless mode, auto injection, and FID detector, or equivalent.
2. 30 meter x 0.25 mm i.d. DB-17MS capillary column (0.25 □m film thickness), Agilent, Catalog #122-4732, or equivalent.
3. Hydrogen generator, Parker Balston, Model 75-33, or equivalent.
4. Zero air generator, Balston, Model 75-83, or equivalent.
5. Analytical balance, Mettler AE-20, or equivalent.
6. Orbital shaker, Lab-Line, or equivalent.
7. Eppendorf autopipettor, VWR, Catalog #53512-500, or equivalent.

Consumables

1. Double tapered glass GC injector liner, Agilent, Catalog # 5181-3315, or equivalent.
2. 20 mL scintillation vials and foil lined caps, VWR, Catalog #66022-004, or equivalent.
3. 2 mL vials with caps, VWR, Catalog #66030-054, or equivalent.
4. 25 mm, 0.45 □m PTFE disk filters, VWR, Catalog #199-2045, or equivalent.
5. Disposable pipette tips, Redi-Tips, 1000 □L Fisher Scientific. Catalog #21-197-8F, or equivalent.
6. 20 mL bottle top dispenser, VWR, Catalog #53501-081, or equivalent.
7. 3 mL disposable syringe fitted with Luer lock tip, VWR, Catalog #BD309586, or equivalent.
8. 10 mL bottle top dispenser, VWR, Catalog #53501-060, or equivalent.

Reagents

1. L,L-Lactide, Purac Biochem, phone# 1-847-634-6330, or equivalent.
2. *Meso*-Lactide, Purac Biochem, phone# 1-847-634-6330, or equivalent.
3. 2,6-Dimethyl-□-pyrone, Aldrich, 99% pure, Catalog #D18340-7, or equivalent.
4. Methylene Chloride, EM Science Omnisolv, VWR, Catalog #EM-DX0831-1, or equivalent.

5. Cyclohexane, EM Science Omnisolv, VWR, Catalog #EM-CX2286-1, or equivalent.
6. Acetone, Reagent Grade, EM Science Omnisolv, VWR, Catalog #EM-AX0120-8, or equivalent.

SAFETY NOTES

1. Methylene Chloride: Health(2), Flammability(1), Reactivity(0)
2. Cyclohexane: Health(1), Flammability(3), Reactivity(0)
3. Acetone: Health(1), Flammability(3), Reactivity(0)
4. 2,6-Dimethyl-□-pyrone: Health(2), Flammability(1), Reactivity(1)
5. Lactides: Health(1), Flammability(1), Reactivity(1)

Specific Hazards

1. Work in a hood to avoid breathing fumes.
2. Keep solvents away from heat or sparks.
3. Wear protective gloves to avoid contact with skin.

PROCEDURE

Preparation of 10,000 ppm Internal Standard Stock Solution

1. Add ~500 mg of 2,6-dimethyl-γ-pyrone into a tared 50 mL volumetric flask. Record the weight to four decimal places. (See Calibration and QC section for D,L and *meso*-lactide standards)
2. Add 25 mL of methylene chloride to the flask.
3. Shake the flask until all the 2,6-dimethyl-γ-pyrone goes into solution.
4. Dilute to volume using methylene chloride.

Preparation of 10,000 ppm Lactide Standard Stock Solution

1. Add ~500 mg of Purac L,L-Lactide into 50 mL volumetric flask. Record the weight to four decimal places. (See Calibration and QC section for *meso*-lactide standard)
2. Add 25 mL of methylene chloride to the flask.
3. Shake the flask until all the L,L-Lactide goes into solution.
4. Dilute to volume using methylene chloride.

Preparation of 10,000 μg Lactide Working Standard Solution

1. Using an Eppendorf autopipettor, accurately transfer 1.0 mL of 10,000 ppm L,L-Lactide standard stock solution to the scintillation vial.
2. Using an Eppendorf autopipettor, accurately transfer 1.0 mL of 10,000 ppm 2,6-dimethyl-γ-pyrone internal standard stock solution to the scintillation vial.
3. Using a bottle top dispenser, add 18 mL of methylene chloride into the scintillation vial (**Vial #1**).
4. Shake **Vial #1** to ensure proper mixing.
5. Using a bottle top dispenser, add 3 mL of acetone to a second 20 mL scintillation vial (**Vial #2**).
6. Using an Eppendorf autopipettor, accurately transfer 1.0 mL of solution from **Vial #1** into **Vial #2**.
7. Using a bottle top dispenser, add 16 mL of cyclohexane into **Vial #2**.
8. Transfer the above solution into a GC autosampler vial using a disposable plastic pipette.
9. Analyze GC autosampler vial contents by GC/FID.

Preparation of PLA Samples

1. Add ~1.0 g of PLA into a 20 mL scintillation vial. Record the weight to four decimal places.
2. Using an Eppendorf autopipettor, accurately transfer 1.0 mL of 10,000 ppm 2,6-dimethyl-□-pyrone internal standard stock solution to the scintillation vial.
3. Using a bottle top dispenser, add 18 mL of methylene chloride into the scintillation vial (**Vial #1**).
4. Shake **Vial #1** until the polymer goes into solution. (Use an orbital shaker @ 300 RPM)
5. Using a bottle top dispenser, add 3 mL of acetone to a second 20 mL scintillation vial (**Vial #2**).
6. Using an Eppendorf autopipettor, accurately transfer 1.0 mL of solution from **Vial #1** into **Vial #2**.
7. Using a bottle top dispenser, add 16 mL of cyclohexane into **Vial #2**.
8. Filter the above solution into a GC autosampler vial using a 3 mL disposable syringe equipped with a 25 mm, 0.45 □m PTFE filter. (Start collecting the solution after purging a few drops to waste.)
9. Analyze GC autosampler vial contents by GC/FID.

Instrument Conditions

1. Carrier Gas: Hydrogen
2. Injector Temperature: 200 °C
3. Injection Volume: 1 □L
4. Injection Type: Auto/Splitless
5. Splitless Valve Time: 1 minute
6. Column Flow Rate: 1.8 mL/minute, set for constant flow.
7. Oven Temperature Program:
    i. Initial Temperature: 50 °C
    ii. Initial Time: 1 minute
    iii. Ramp Rate: 25 °C/minute
    iv. Final Temperature: 320 °C
    v. Final Time: 5 minutes
8. Detector temperature: 335 °C

CALIBRATION AND QC

- One point calibration can be used for this method.
- Standard stock solutions can be stored for up to a month. If this test is not performed routinely, fresh standard stock solutions should be prepared and used.
- L,L-lactide standard of high known purity is used to calibrate this method. To determine the retention time of *meso*-lactide stereoisomer, a standard should be purchased and analyzed using established method. It should be noted that although there are three lactide stereoisomers, only two lactide peaks are detected using this GC method. The earliest eluting peak is due to *meso*-lactide and the second, later-eluting peak contains co-eluting D,D-/L,L-lactide enantiomers (henceforth designated D,L-lactide). When determining relative response factors, we calculate relative response factor for D,L-lactide and assume that the response factors for all three lactide stereoisomers are identical.
- A check sample should be analyzed once every ten injections. A PLA pellet sample with known concentration of residual lactide can be used as the check sample.
- The results for the check sample should fall within three standard deviations of the average results before actual samples are analyzed.

CALCULATIONS

1. Calculate the relative response factors for the standards according to the following equation (keep in mind that D,D-lactide and L,L-lactide enantiomers co-elute using DB-17MS capillary column for the separation). IS=Internal Standard.

$$\text{RRF} = \left(\frac{\text{Peak Area of D,L – Lactide Standard}}{\text{Amount (g) of D,L – Lactide}} \right) \times \left(\frac{\text{Amount (g) of IS}}{\text{Peak Area of IS}} \right)$$

2. Weight of D,L-lactide (g) in sample can be calculated accordingly

$$\text{D,L - Lactide (g)} = \left(\frac{\text{Peak Area of D,L – Lactide in Sample}}{\text{RRF}} \right) \times \left(\frac{\text{Amount (g) of IS}}{\text{Peak Area of IS}} \right)$$

3. Calculate the wt% D,L-lactide in the sample according to the following equation

$$\text{wt \% D,L – Lactide in sample} = \left(\frac{\text{D,L – Lactide (g)}}{\text{SampleWeight (g)}} \right) \times 100$$

4. Weight of *meso*-lactide (g) in sample can be calculated accordingly

$$\text{meso - Lactide (g)} = \left(\frac{\text{Peak Area of } meso\text{ – Lactide in Sample}}{\text{RRF}} \right) \times \left(\frac{\text{Amount (g) of IS}}{\text{Peak Area of IS}} \right)$$

5. Calculate the wt% *meso*-lactide in the sample according to the following equation

$$\text{wt \% meso – Lactide in sample} = \left(\frac{meso\text{ – Lactide (g)}}{\text{SampleWeight (g)}} \right) \times 100$$

6. Add the results for D,L-lactide and *meso*-lactide to obtain total wt% residual lactide stereoisomers.

Instrument Maintenance

1. Replace old septum with new one after about 100 injections.
2. Before analyzing any samples, condition the column by manually ramping oven temperature to 320 °C and hold at 320 °C for 10 minutes.
3. Periodically clean FID detector. Place collector, flame jet, and insulators into glass beaker with methanol and sonicate for 15 minutes.
4. If all of the above instrument maintenance procedures do not improve instrument performance, cut 4-6 inches of capillary column off the injector side.
5. Change GC inlet liner and gold seal as needed. Visually inspect once a week. If dark colored material is present, replace GC inlet liner and/or gold seal.

VALIDATION INFORMATION

Linearity

This method has demonstrated linearity over the 0.1-5 wt% residual lactide stereoisomer concentration range with R^2 values equal to or greater than 0.9999. Found in **Figure 1** is a representative gas chromatogram for PLA sample analyzed using this method. See **Figures 2** and **3** for representative calibration curves for *meso*- and D,L-lactide peaks.

Figure 1

Representative Gas Chromatogram for PLA Sample Analyzed Using Established Method

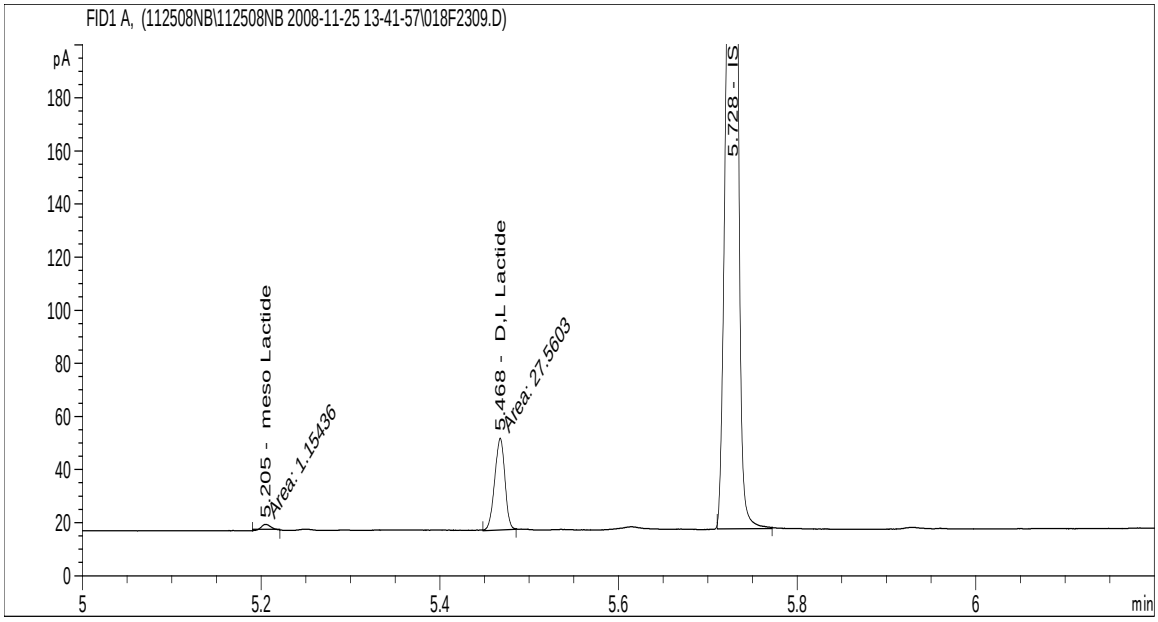


Figure 2: Calibration Curve for *Meso*-Lactide Peak (5.205 min retention time in **Figure 1**)

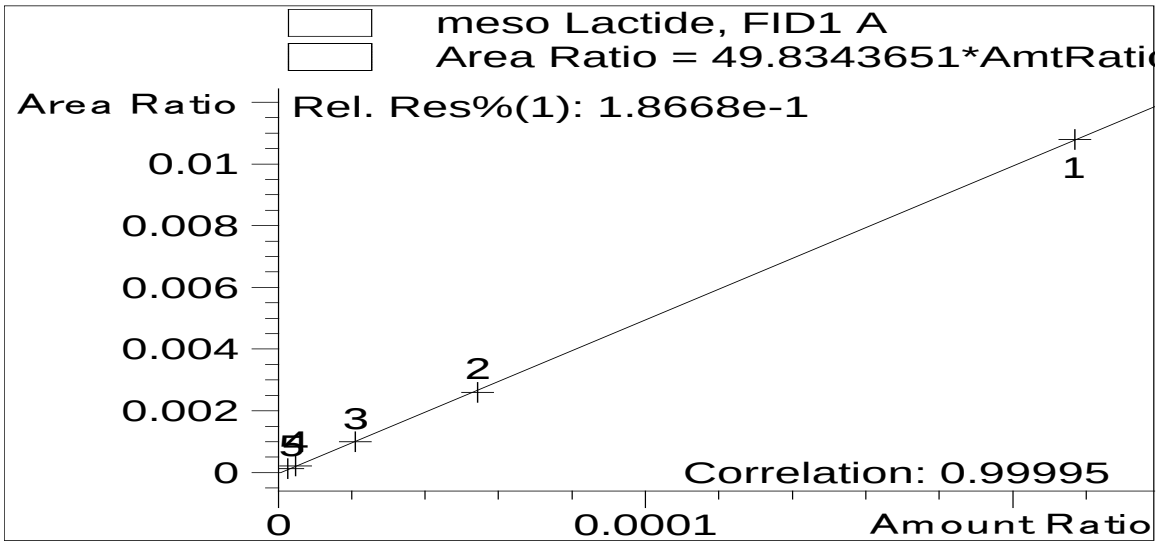
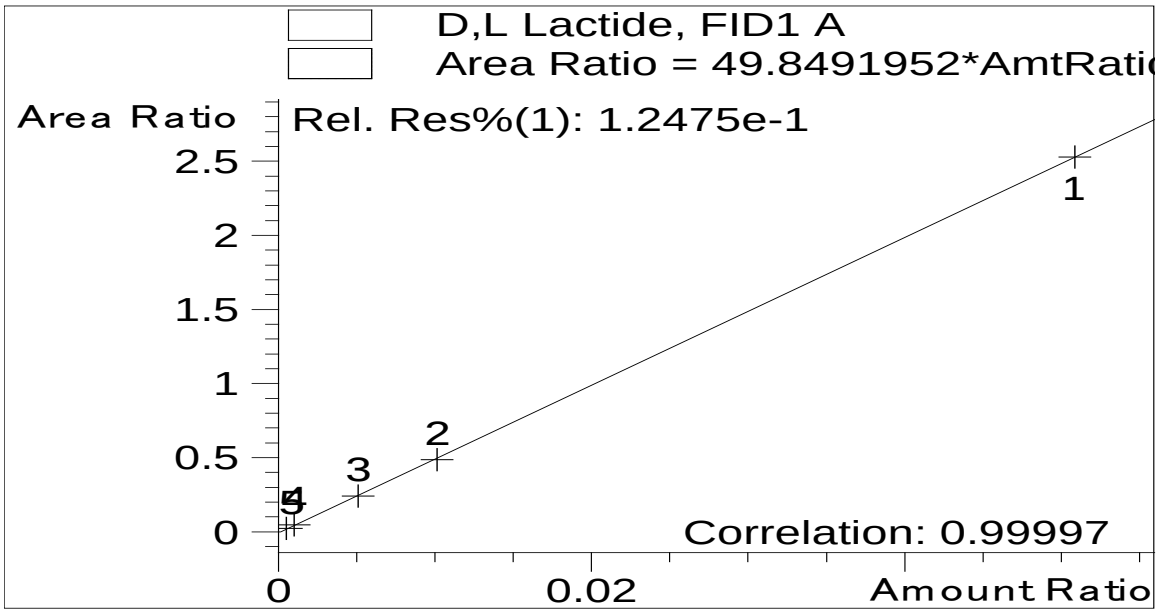


Figure 3: Calibration Curve for D,L-Lactide peak (5.468 min retention time in **Figure 1**)



Instrument and Method Precision

An "In Spec" (i.e. <0.3 wt% residual lactide) PLA pellet sample was analyzed to determine instrument and method precision. In the **Tables** presented below, Residual Lactide (RL) designates the sum of quantified *meso*- and D,L-lactide isomers.

Instrument Precision

One preparation of PLA pellet sample was injected 10 times from the same vial to evaluate instrument precision. Instrument precision of 0.71 %RSD was evaluated. Experimental data are presented in **Table 1.**

Table 1: Instrument Precision for PLA Pellet Sample

Injection #	wt% RL
1	0.188
2	0.190
3	0.187
4	0.188
5	0.189
6	0.187
7	0.190
8	0.188
9	0.189
10	0.191
Avg	**0.189**
Std Dev	**0.001**
%RSD	**0.710**

Method Precision

Ten separate preparations of PLA pellet sample used to evaluate instrument precision were injected once to evaluate method precision. Method precision of 1.9 %RSD was evaluated. Experimental data are presented in **Table 2.**

Table 2: Method Precision for PLA Pellet Sample

Preparation #	wt% RL
1	0.192
2	0.183
3	0.179
4	0.184
5	0.186
6	0.188
7	0.185
8	0.189
9	0.187
10	0.188
Avg	**0.186**
Std Dev	**0.004**
%RSD	**1.937**

Method Accuracy

Method accuracy was assessed by determining recoveries for L,L-lactide spiked at three different concentrations into recrystallized PLA pellet sample. The PLA pellets subjected to recrystallization are the same pellets analyzed for instrument and method precision (*vide supra*). Residual lactide concentration in this PLA sample decreased from 0.19 to 0.022 wt% after recrystallization. Lactide was spiked to recrystallized PLA sample at concentrations equivalent to 0.1 (spike level 1), 0.2 (spike level 2) and 0.3 (spike level 3) wt%. Each lactide spike concentration was prepared in triplicate. Lactide spike recoveries in the 95-100% range were evaluated. The experimental data for lactide spike recovery experiments are presented in **Table 3**.

Table 3: Recoveries for Lactide Spiked to Recrystallized PLA Pellets

Sample Identification	RL in Recrystallized PLA, g	Lactide Spiked, g	Lactide Detected, g	%Recovery
Spike Level 1; Prep A	0.00022	0.001021	0.00123	99.1
Spike Level 1; Prep B	0.00022	0.001021	0.00123	99.1
Spike Level 1; Prep C	0.00022	0.001021	0.00129	103.9
			Avg	**100.7**
Spike Level 2; Prep A	0.00022	0.002042	0.00215	95.0
Spike Level 2; Prep B	0.00022	0.002042	0.00216	95.5
Spike Level 2; Prep C	0.00022	0.002042	0.00216	95.5
			Avg	**95.3**
Spike Level 3; Prep A	0.00022	0.003063	0.00311	94.7
Spike Level 3; Prep B	0.00022	0.003063	0.00309	94.1
Spike Level 3; Prep C	0.00022	0.003063	0.00315	95.9
			Avg	**94.9**

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