Determination of *cis,cis*- and *cis,trans*-Muconic Acid from Biological Conversion

Laboratory Analytical Procedure (LAP)

Issue Date: September 4, 2019

Brenna A. Black, William E. Michener, Courtney E. Payne, and Gregg T. Beckham

National Renewable Energy Laboratory

NREL is a national laboratory of the U.S. Department of Energy
Office of Energy Efficiency & Renewable Energy
Operated by the Alliance for Sustainable Energy, LLC

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at www.nrel.gov/publications.

Contract No. DE-AC36-08GO28308

Technical Report
NREL/TP-5100-74473
September 2019

Determination of *cis,cis*- and *cis,trans*-Muconic Acid from Biological Conversion

Laboratory Analytical Procedure (LAP)

Issue Date: September 4, 2019

Brenna A. Black, William E. Michener, Courtney E. Payne, and Gregg T. Beckham

National Renewable Energy Laboratory

Suggested Citation
Black, Brenna A., William E. Michener, Courtney E. Payne, and Gregg T. Beckham. 2019. *Determination of cis,cis- and cis,trans-Muconic Acid from Biological Conversion*. Golden, CO: National Renewable Energy Laboratory. NREL/TP-5100-74473. https://www.nrel.gov/docs/fy19osti/74473.pdf.

NREL is a national laboratory of the U.S. Department of Energy
Office of Energy Efficiency & Renewable Energy
Operated by the Alliance for Sustainable Energy, LLC

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at www.nrel.gov/publications.

Contract No. DE-AC36-08GO28308

Technical Report
NREL/TP-5100-74473
September 2019

National Renewable Energy Laboratory
15013 Denver West Parkway
Golden, CO 80401
303-275-3000 • www.nrel.gov

NOTICE

This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office. The views expressed herein do not necessarily represent the views of the DOE or the U.S. Government.

Cover Photos by Dennis Schroeder: (clockwise, left to right) NREL 51934, NREL 45897, NREL 42160, NREL 45891, NREL 48097, NREL 46526.

DISCLAIMER

The Muconic Acid Analytical Methods (Methods) are provided by the National Renewable Energy Laboratory (NREL), which is operated by Alliance for Sustainable Energy, LLC (Alliance) for the U.S. Department of Energy (DOE). These methods were developed and written for commercial research and educational use only.

Access to and use of these Methods shall impose the following obligations on the user. The user is granted the right, without any fee or cost, to use, copy, modify, alter, enhance, and distribute these Methods for any purpose whatsoever, except commercial sales, provided that this entire notice appears in all copies of the Methods. The user agrees to credit NREL/Alliance in any publications that result from the use of these Methods. The user also understands that NREL/Alliance is not obligated to provide the user with any support, consulting, training, or any training or assistance of any kind with regard to the use of these Methods or to provide the user with any updates, revisions, or new versions.

THESE METHODS ARE PROVIDED BY NREL/Alliance "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE DISCLAIMED. IN NO EVENT SHALL NREL/Alliance/DOE BE LIABLE FOR ANY SPECIAL, INDIRECT, OR CONSEQUENTIAL DAMAGES OR ANY DAMAGES WHATSOEVER, INCLUDING BUT NOT LIMITED TO, CLAIMS ASSOCIATED WITH THE LOSS OF DATA OR PROFITS, WHICH MAY RESULT FROM AN ACTION IN CONTRACT, NEGLIGENCE, OR OTHER TORTIOUS CLAIM THAT ARISES OUT OF OR IN CONNECTION WITH THE ACCESS, USE, OR PERFORMANCE OF THESE METHODS.

1. Introduction

1.1 Muconic acid is an important platform molecule for production of bio-based polymers. To date, *cis,cis*-muconic acid (*cc*MA) has been produced from multiple types of carbon sources including carbohydrates and aromatic compounds via cultivation with genetically engineered microbes [1]. Accurate analysis of muconic acid has previously been a challenge due to *cc*MA isomerization into *cis,trans*-muconic acid (*ct*MA). Isomerization of muconic acid from *cc*MA to *ct*MA appears to be initiated by a drop in pH (below 7) and/or with the addition of heat. Heat also leads to the creation of side products such as muconolactone (Mlac) and tetrahydrofuro[3,2-*b*]furan-2,5-dione (Lac2) (see Appendices Figure 1).

1.2 Generally, the complications that arise with the analysis of muconic acid are twofold: (1) the inability of common chromatographic techniques to resolve *cc*MA and *ct*MA and (2) lack of suitable standards. Common methods for the analysis of biological acids, such as high-performance liquid chromatography (HPLC) methods using ionic hydrogen columns (i.e., Phenomenex Rezex RFQ-Fast Acid or BioRad Aminex HPX-87H), are not suitable to resolve both *cc*MA and *ct*MA. The current lack of a commercially available *ct*MA analytical standard makes it difficult to determine detector response. Accurate chromatographic quantitation (i.e., gas chromatography, liquid chromatography [LC], etc.) requires a detector response from varying concentrations of pure standards to construct individual compound calibration curves for each isomer. In addition to having differing detector responses, muconic acid isomers also differ in water solubility (*cc*MA = ~1 g/L and *ct*MA = 5.2 g/L) [2], which further amplifies quantitative inaccuracy in analytical methods that fail to analyze both isomers separately. Each of these issues results in less accurate overall muconic acid quantitation in biological samples.

1.3 This procedure accurately and separately determines the concentrations of *cc*MA and *ct*MA representing total muconic acid in biological conversion samples. In this procedure, a purchasable authentic *cc*MA standard is used for detector response and biological sample quantitation of *cc*MA by HPLC with diode array detection (DAD) and refractive index detection (RID). Separately, this procedure provides a method to convert the purchasable *cc*MA to *ct*MA to produce the *ct*MA standard isomer used for detector response and quantitation by the same LC methods. The chromatography methods presented here have been tested to ensure that they do not induce further isomerization of *cc*MA.

1.4 This procedure also addresses matrix effects, whether media- or substrate-based, on muconic acid isomers regarding accurate quantitation of total muconic acid. A matrix media spike (MMS) is implemented as a confirmation of dependable analysis, or if necessary, when sample dilutions are required.

2. Scope

2.1 This procedure is used to separately quantify *cc*MA and *ct*MA as the total sum of muconic acid present in the liquid fraction of pretreated biological samples.

2.2 Analysis of *trans, trans*-muconic acid (*tt*MA) is outside the scope of this work, since *tt*MA production has not be observed in biological samples. The pathway to produce *tt*MA from *cc*MA differs from the lactonization pathway, and intermediate compounds from alternative pathways have not been studied with application to the methods presented in this procedure.

2.3 Sample matrices appropriate to methods presented in this procedure include M9 minimal media, herbaceous feedstock-based alkaline pretreated liquor [3], base catalyzed depolymerized liquor [4], and deacetylated mechanically refined liquor with enzymatic hydrolysis [5,6]. These methods are not appropriate for whole slurry or acidic samples, only the liquid fractions of the aforementioned pretreatment types.

2.4 This procedure is appropriate for samples containing muconic acid, glucose, gluconate, 2-ketogluconate, and aromatic monomers and aromatic dimers present in the previously described pretreated lignin matrices. Any sample and/or matrix containing interfering components (such as co-eluting constituents) must be further investigated.

2.5 All analyses should be performed in accordance with an appropriate laboratory-specific quality assurance plan (QAP).

3. Terminology

3.1 *Calibration Standard*: A set of standards, each at a known concentration, that are used to determine a detector response. The calibration standards must include the analytes of interest and be run in series with a sample set. The detector response can then be used to predict the concentration of an analyte in a sample.

3.2 *Check Verification Standard (CVS):* An independent standard prepared from a different stock solution at a different concentration than the calibration standards. The concentration of the CVS should be set in the middle of the chosen calibration range and is used to ensure the calibration remains accurate throughout the entirety of the sample run. Additional CVS concentrations at the lower or higher end of the calibration curve can also be run to ensure accuracy of sample respective concentrations.

3.3 *Media Matrix Spike (MMS):* The addition of the analyte(s) of interest at a known concentration into a media blank. This standard is used to evaluate the effects of the sample matrices on the performance of the analytical method. Extreme levels of pH, ionic strength, or the concentration of naturally occurring organic matter in the sample can enhance or decrease the detector response, resulting in unreliable analyte quantitation.

4. Significance and Use

4.1 This procedure is used to determine total muconic acid in the liquid fraction pretreated biological samples. This procedure may be used in conjunction with other methods to characterize and track other metabolites and sources of carbon such as the NREL laboratory analytical procedures "Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples" and "Determination of Structural Carbohydrates and Lignin in Biomass."

5. Interferences

5.1 With samples that have been exposed to acidic conditions (pH below 7) for prolonged periods of time, any *ct*MA present will likely undergo further conversion into lactones along the lactonization pathway, resulting in an inaccurate total muconic acid quantitation. The addition of heat reduces the reaction time to lactonization. Lactones are not quantified in this method and cannot currently be quantified as there are no available standards. Lactone standards cannot be produced in the same manner as *ct*MA, due to the reaction equilibrium of Mlac and Lac2 (see Appendices Figure 1).

5.2 Biological samples exposed to irradiation with ultraviolet light, iodine in acetonitrile, or specific catalysts such as palladium on carbon, produce *tt*MA in a separate reaction pathway from the lactonization pathway. Analysis of *tt*MA is outside the scope of this work.

5.3 In this method, muconic acid isomers appear to be sensitive to specific media matrices, often appearing as double peaks. Media matrix effects should be tested by using an MMS as previously defined. Dilution of samples may be necessary.

5.4 This method is not appropriate if analytes from sample and/or media co-elute with either muconic acid isomer.

6. Apparatus

6.1 Analytical balance with precision to 0.1 mg

6.2 Water bath set to 60 ± 3°C

6.3 HPLC system equipped with a refractive index or diode array detector and the following columns:

6.3.1 Reverse phase column with non-polar end capping: Phenomenex Luna C18(2) column, 100 Å, 5 µm, 150 × 4.6 mm (Phenomenex #00F-4252-E0)

6.3.2 Guard column compatible with column: Phenomenex SecurityGaurd cartridges (Phenomenex #AJ0-4287) and SecurityGaurd cartridge kit (Phenomenex #KJ0-4282), or equivalent

7. Reagents and Materials

7.1 Reagents

7.1.1 *cis,cis*-muconic acid (*cis,cis*-2,4-hexadienedioic acid, ≥97% pure)

7.1.2 Second *cis,cis*-muconic acid standard, as listed above, from a different source (manufacturer or lot), to be used to prepare CVS

7.1.3 Sodium hydroxide 10N, ACS reagent grade (≥30% w/w)

7.1.4 Water, HPLC grade, 0.2 µm filtered

7.1.5 Methanol, HPLC grade

7.1.6 Formic acid, ACS reagent grade (≥98%)

7.2 Materials

7.2.1 Vials, amber, borosilicate, twist-top with teflon septa, 40-mL fill volume (Environmental Sampling Supply, #0040-0400-QC, REQUIRED: The procedure was developed around the use of these vials and their specific heat transfer.)

7.2.2 Crimp-top cap, polytetrafluoroethylene/rubber septa, 11 mm (Agilent Technologies, #5183-4498, REQUIRED)

7.2.3 Autosampler vials, amber, silanized, crimp-top, 2-mL fill volume

7.2.4 Repeater pipette with a 50-mL positive displacement tip or equivalent

7.2.5 Air displacement pipettes, 20–200 µL, 100–1,000 µL, and compatible tips

7.2.6 Disposable syringes, 3 mL, fitted with 0.2-µm nylon syringe filters

8. Environmental Safety and Health Considerations and Hazards

8.1 Sodium hydroxide is corrosive and should be handled with care.

8.2 Methanol is a slight health hazard and is moderately flammable.

8.3 Formic acid is toxic, flammable, and should be handled with care.

8.4 Follow all applicable chemical handling procedures such as those provided by material safety data sheets.

9. Sampling, Test Specimens, and Test Units

9.1 Care must be taken to ensure a representative sample is used for analysis. The sample for analysis should be at room temp. If sample was previously frozen and precipitate visible, samples need to be sonicated to re-dissolve the precipitate. Once precipitate is no longer visible, sample should be homogenized through vortexing and filtered through a 0.2-µm nylon syringe filter prior to analysis.

9.2 Limit sample contact with heat and prolonged exposure to a pH below 7 to lessen the possibility of muconic acid conversion to lactones. Samples should ideally be neutralized and stored at -20°C.

9.3 Avoid sample contact with polar protic solvents, such as dimethyl sulfoxide and acetonitrile, as these at solvents promote lactonization of muconic acid.

10. Procedure

10.1 Prepare solutions and standards as follows:

10.1.1 Prepare a water:methanol:formic acid solution [80:20:0.16, v/v/v] for use as an HPLC mobile phase. In a 1-L volumetric flask add 799.2 mL of HPLC-grade water. Add 1.6 mL of formic acid (≥98%) and bring to volume with HPLC-grade methanol. Filter through a 0.2-µm filter and degas before use. This solution may be prepared ahead of time and stored for up to two months. It is suggested that enough mobile phase is made for a complete analysis or elution times may vary.

10.1.2 Prepare a sodium hydroxide solution (0.05% v/v) for sample dilution and standard preparation. Prepared by adding 66 µL 10N sodium hydroxide with an air displacement pipette to 39.934 mL of filtered HPLC-grade water measured using a repeater pipette. This solution may be scaled if necessary.

10.1.3 Prepare the *cc*MA stock standard by weighing 40.0 ± 0.5 mg of the *cc*MA standard into a 40-mL amber vial and record the weight of the standard to the nearest 0.1 mg. Add an appropriate volume of 0.05% v/v sodium hydroxide solution using a repeater pipette to make up exactly 1.0 mg/mL solution and mix well (vigorous shaking for approximately 1 hour required for solubilization.) The muconic acid should be completely dissolved before using or the concentration of the standard will be unknown. Record the date of preparation, concentration, and any other pertinent information on the vial and store sealed at 4°C for up to 4 months (stability study ongoing).

10.1.4 Prepare the *ct*MA stock standard by preheating a water bath to 60 ± 3°C. Weigh exactly 40.0 mg of the *cc*MA standard into a 40-mL amber vial (vial REQUIRED as ordered per Step 7.2.1; vial variation will lead to heat transfer difference and the reaction time will either lead to incomplete *ct*MA formation or lactone formation). Record the weight of the standard to the nearest 0.1 mg. Add 39.934 mL HPLC-grade water or similar using a repeater pipette and mix well. Record the concentration of the standard, date of preparation, and any other pertinent information on the vial. Seal the standard with compatible vial top and place into the water bath so that the liquid in the vial is completely submerged for 2 hours. Shake every 15 minutes (use personal protective equipment as necessary). After 2 hours, immediately add 66 µL 10N sodium hydroxide using an air displacement pipette and mix. Store the sealed vial at 4°C for up to 4 months (stability study ongoing).

10.1.5 Prepare the media matrix spikes separately by making up a 100 µg/mL *cc*MA MMS and 100 µg/mL *ct*MA MMS. For the *cc*MA MMS, add 100 µL of the *cc*MA 1 mg/mL standard solution (Step 10.1.3) to 900 µL sample media using appropriate volume air displacement pipettes. For the *ct*MA MMS, add 100 µL of the *ct*MA 1 mg/mL standard solution (Step 10.1.4) to 900 µL sample media using appropriate volume air displacement pipettes. DO NOT mix *cc*MA and *ct*MA standards together in one matrix spike. The *cc*MA standard has potential to convert to *ct*MA due to issues such as temperature abuse, lack of pH control, prolonged storage, etc., and this will not be observed if mixed. Matrix spikes should be prepared and used for analysis within 24 hours or they will need to be remade. Standards cannot be stored to prevent *cc*MA conversion within the matrix/media over time.

10.2 Prepare a series of calibration standards for *cc*MA and *ct*MA, and the CVS as follows:

10.2.1 Prepare a series of calibration standards containing the *cc*MA stock standard prepared above referring to Table 1 (Step 10.2.4) for suggested concertation ranges. Dilute standards, using the 0.05% v/v sodium hydroxide solution, into 2-mL vials labeled with standard type, level, and date. Fully prepare each standard level before moving on to the next: use appropriate volume air displacement pipettes. Seal the vials.

10.2.2 Use a five-point calibration, unless both detectors (RID and DAD) are used, then a nine-point calibration is recommended. *cc*MA and *ct*MA elute approximately 2 minutes apart as outlined in Table 2.

10.2.3 Use the calibration response factor obtained from the resulting linear calibration curve for *cc*MA and only work up data when the calibration quality of correlation is 0.995 or better. Response factors will differ between *cc*MA and *ct*MA; it is important to use the respective calibration curves to determine quantitation.

10.2.4 Information for standard concentrations and retention times.

Table 1. Standards Prepared Using *cc*MA and *ct*MA from Steps 10.1.3 and 10.1.4, respectively

Standard Level Concentration (µg/mL)	Applicable Detector	
0.5	DAD	
1	DAD	
5	DAD	
10	DAD	
50	DAD	RID
100	DAD	RID
200	DAD	RID
500		RID
1,000		RID
CVS 75	DAD	
CVS 300		RID

Table 2. Approximate Chromatographic Retention Times of *cc*MA and *ct*MA

Standard	Approximate elution time (min)
*cc*MA	6–7
*ct*MA	8–9

10.2.5 Prepare a series of calibration standards containing the *ct*MA stock standard prepared above referring to Table 1 (Step 10.2.4) for suggested concentration ranges. Dilute standards using the 0.05% v/v sodium hydroxide solution into 2-mL vials labeled with standard type, level, and date. Fully prepare each standard level before moving on to the next: use appropriate volume air displacement pipettes. Seal the vials.

10.2.6 Use a five-point calibration, unless both detectors (RID and DAD) are used, then a nine-point calibration is recommended.

10.2.7 Use the calibration response factor obtained from the resulting linear calibration curve for *ct*MA and only work up data when the calibration quality of correlation is 0.995 or better. Response factors will differ between *cc*MA and *ct*MA; it is important to use the respective calibration curves to determine quantitation.

10.2.8 Prepare an independent CVS for each set of calibration standards. Use reagents from a source or lot other than that used in preparing the calibration standards. Prepare the CVS at a concentration that falls in the middle of the validated range of the calibration curve. The CVS should be analyzed on the HPLC after each calibration set and should be included in the sequence table every 10 to 20 samples and at the end of the run to ensure the calibration holds throughout the analysis. The CVS is used to verify the quality and stability of the calibration curve(s) throughout the run.

10.3 Analyze the biological samples for *cc*MA and *ct*MA as follows:

10.3.1 Analyze MMS using calibration curves (Steps 10.2.1 and 10.2.5) prior to biological sample preparation and analysis (Step 10.3.3). The MMS is used for determining the necessary sample dilution to lessen matrix effect on the muconic acid isomers. Analysis of the MMS should determine the expected undiluted quantitation (100 µg/mL) of both *cc*MA and *ct*MA. If the theoretical quantitation does not match the observed value or if irregular peak shape of either muconic acid isomer occurs, perform a dilution to minimize the matrix effects. Dilute MMS using the sodium hydroxide solution (Step 10.1.2).

10.3.2 Any dilution necessary must be carefully balanced because over dilution will decrease the concentration of muconic acid when applied to the samples, possibly below the limit of quantitation. The suggested dilution factor is five for a media matrix with a M9 composition.

10.3.3 Prepare and analyze the biological samples by applying the appropriate dilution to each sample as determined in Step 10.3.1 to reduce matrix effects on muconic acid. The suggested dilution factor is five for a media matrix with a M9 composition. Filter each sample using 0.2-µm nylon filters.

10.3.4 Analyze the calibration standards, CVS, MMS, and samples using a C18(2) column, 100 Å, 5 µm, 150 × 4.6 mm.

HPLC Conditions:
Injection Volume: 8 µL
Mobile Phase: water:methanol:formic acid (80:20:0.16, v/v/v), isocratic
Flow Rate: 0.65 mL/min
Column Temperature: 45°C
RID Detector Temperature: 45°C
DAD Monitoring: 265 nm
Run Time: 15 minutes

11. Calculations

11.1 Create a calibration curve for *cc*MA to be quantified using linear regression. From this curve, determine the concentration in mg/mL of the component present in the samples analyzed by HPLC, correcting for dilution if required.

11.2 Create a calibration curve for *ct*MA to be quantified using linear regression. From this curve, determine the concentration in mg/mL of the component present in the samples analyzed by HPLC, correcting for dilution if required.

11.3 Calculate and record the amount of each CVS recovered following HPLC analysis.

$$\%CVS\ recovery = \frac{conc.detected\ by\ HPLC, mg/mL}{known\ conc.of\ standard, mg/mL} \times 100$$

11.4 Export the *cc*MA and *ct*MA concentrations, as µg/mL for each individual isomer, from the LC software and calculate the total muconic acid as µg/mL in each sample

$$Total\ muconic\ acid\ = Amount_{\ Measured\ cis,cis} + Amount_{Measured\ cis,trans}$$

11.5 To report or calculate the relative percent difference (RPD) between two samples, use the following calculation:

$$RPD = \left(\frac{(X_1 - X_2)}{X_{mean}}\right) \times 100$$

Where:

X_1 and X_2 = measured values
X_{mean} = the mean of X_1 and X_2

11.6 To report or calculate the root mean square (RMS) deviation or the standard deviation (stdev) of the samples, use the following calculations.

First find the RMS of the sample using:

$$RMS = x_m = mean = \sqrt{\left(\frac{\sum_1^n x}{n}\right)^2}$$

Then find the root mean square deviation, or stdev, using:

$$RMSdeviation = \sigma = stdev = \sqrt{\frac{\sum_1^n (X_i - X_m)^2}{n}}$$

Where:

x_m = the root mean square of all x values in the set
n = number of samples in set

x_i = a measured value from the set

12. Report Format

12.1 Report each muconic acid isomer and the total muconic acid in each sample as a concentration in µg/mL. RPD and RMS deviation may also be reported.

13. Precision and Bias

13.1 Precision and bias need to be determined by data quality objectives and laboratory-specific QAP.

14. Quality Control

14.1 *Reported Significant Figures:* Figures need to be determined by data quality objectives and laboratory-specific QAP.

14.2 *Replicates:* It is recommended that the samples be run in triplicate to verify reproducibility on differing analytical instruments.

14.3 *Blank:* 0.05% sodium hydroxide solution blank and media blank are used to ensure no co-eluting peaks and that there are no detectable compounds present in the media or sodium hydroxide solution due to contamination upon preparation.

14.4 *Relative Percent Difference Criteria:* Criteria need to be determined by data quality objectives and laboratory-specific QAP.

14.5 *Calibration Verification Standard: cc*MA and *ct*MA should be independently produced from a different stock and a different concentration to that of the standards used in the calibration curve.

14.6 *Sample Size:* A sample volume of greater than 1 mL is required for filtering, possible dilutions, and proper injection from a standard 2-mL LC vial. If less than 1 mL sample volume is available, vial inserts may be used.

14.7 *Sample Storage:* It is recommended that all samples be neutralized or brought up to a pH higher than 7, sealed, and stored in a freezer (-20°C or -80°C). Samples have been tested and determined stable with storage up to one month.

14.8 *Standard Preparation:* Standards and CVS should be prepared using 0.05% sodium hydroxide solution as described in the procedure.

14.9 *Standard Storage:* Muconic acid standards should be stored upright in a fridge (4°C) until required. Stability studies are ongoing; however, samples have been tested and determined stable up to four months.

14.10 *Definition of a Batch:* A batch is any number of samples that are analyzed and recorded together. The maximum size of a batch will be limited by the equipment constraints.

15. Appendices

15.1 Figure of the muconate lactonization pathway:

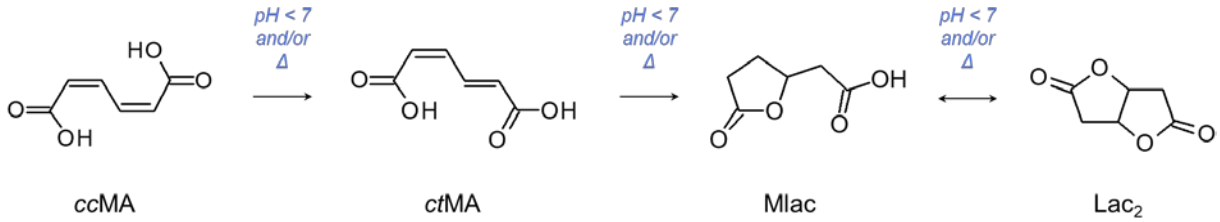

Figure 1. Muconate lactonization pathway utilized to produce *ct*MA standard and possible in biological samples. (*cc*MA = *cis,cis*-muconic acid; *ct*MA = *cis,trans*-muconic acid; Mlac = muconolactone; Lac2 = tetrahydrofuro[3,2-*b*]furan-2,5-dione)

16. References

[1] Xie, N.-Z., H. Liang, R.-B. Huang, and P. Xu, "Biotechnological production of muconic acid: current status and future prospects," *Biotechnology Advances* 32, no. 3 (2014): 615–622, https://doi.org/10.1016/j.biotechadv.2014.04.001.

[2] Carraher, J. M., T. Pfennig, R. G. Rao, B. H. Shanks, and J.-P. Tessonnier, "cis,cis-Muconic acid isomerization and catalytic conversion to biobased cyclic-C6-1,4-diacid monomers," *Green Chemistry* 19, no. 13 (2017): 3042–3050, https://doi.org/10.1039/C7GC00658F (2017).

[3] Karp, E. M., B. S. Donohoe, M. H. O'Brien, P. N. Ciesielski, A. Mittal, M. J. Biddy, and G. T. Beckham, "Alkaline pretreatment of corn stover: bench-scale fractionation and stream characterization," *ACS Sustainable Chemistry and Engineering* 2, no. 6 (2014): 1481-1491, https://doi.org/10.1021/sc500126u.

[4] Katahira, R., A. Mittal, K. McKinney, X. Chen, M. P. Tucker, D. K. Johnson, and G. T. Beckham, "Base-catalyzed depolymerization of biorefinery lignins," *ACS Sustainable Chemistry and Engineering* 4, no. 3 (2016): 1474-1486, https://doi.org/10.1021/acssuschemeng.5b01451.

[5] Salvachúa, D., R. Katahira, N. S. Cleveland, P. Khanna, M. G. Resch, B. A. Black, S. O. Purvine, E. M. Zink, A. Prieto, M. J. Martínez, A. T. Martínez, B. A. Simmons, J. M. Gladden, and G. T. Beckham, "Lignin depolymerization by fungal secretomes and a microbial sink," *Green Chemistry* 18, no. 22 (2016): 6046-6062, https://doi.org/10.1039/C6GC01531J.

[6] Chen, X., J. Shekiro, R. Elander, and M. Tucker, "Improved xylan hydrolysis of corn stover by deacetylation with high solids dilute acid pretreatment," *Industrial and Engineering Chemistry Research* 51, no. 1 (2011): 70-76, https://doi.org/10.1021/ie201493g.