Analysis of products from deconstructed nylon-6 by UHPLC-MS/MS (dMRM) V.1

Kelsey J. Ramirez[1,2], Morgan A Ingraham[1,2], Elizabeth L. Bell[1,2], Gregg T. Beckham[1,2]

[1]Renewable Resources and Enabling Sciences Center, National Renewable Energy Laboratory, Golden, CO, USA;
[2]BOTTLE Consortium, Golden, CO, USA

Kelsey J. Ramirez

VERSION 1

JAN 07, 2024

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.kxygx331dg8j/v1

Protocol Citation: Kelsey J. Ramirez, Morgan A Ingraham, Elizabeth L. Bell, Gregg T. Beckham 2024. Analysis of products from deconstructed nylon-6 by UHPLC-MS/MS (dMRM). **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx331dg8j/v1

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

DISCLAIMER

This protocol is for research purposes only.

ABSTRACT

An analysis method was developed for the quantitation of products produced by deconstruction of nylon-6 utilizing ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) detection. This method employs reverse phase chromatography and dynamic multiple reaction monitoring (dMRM) in positive ion mode using electrospray ionization (ESI).

GUIDELINES

This protocol utilizes an ultra-high pressure liquid chromatography tandem mass spectrometer (UHPLC-MS/MS) system manufactured by Agilent Technologies as referenced in 'Materials'. A similar liquid chromatography tandem mass spectrometry system can be utilized however, some parameter nomenclature may deviate depending on the manufacturer.

MATERIALS

Reagents:

- Methanol Optima **Fisher Scientific Catalog # A454SK**
- Formic acid 98 % pure **Thermo Scientific Catalog # AC147932500**

Created: Aug 21, 2023

Last Modified: Jan 07, 2024

PROTOCOL integer ID: 86732

Keywords: mass spectrometry, nylonase, nylon-6 deconstruction, enzymatic deconstruction, UHPLC-MS/MS of nylon-6 products

Funders Acknowledgement:
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. This work was performed as part of the BOTTLE™ Consortium and was supported by Office of Energy Efficiency and Renewable Energy (EERE), Bioenergy Technologies Office (BETO), and Advanced Materials and Manufacturing Technologies Office (AMMTO).
Grant ID: DE-AC36-08GO28308.

6-Aminocaproic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2504**

6-(6-aminohexanamido)hexanoic acid **Advanced ChemBlocks Catalog #P51557**

6-(6-(6-Aminohexanamido)hexanamido)hexanoic Acid **Toronto Research Chemicals Inc Catalog #A618823-100MG**

ε-Caprolactam **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C2204-500G**

18-Diaza-29-diketocyclotetradecane **Toronto Research Chemicals Inc Catalog #D417643**

Materials:

syringe filters for aqueous matrices-

Equipment

syringe filters, 13mm nylon membrane	NAME
syringe filter	TYPE
Cytiva	BRAND
4550	SKU
https://www.cytivalifesciences.com/en/us/shop/lab-filtration/syringe-filters-non-sterile/nylon-non-sterile-syringe-filters/acrodisc-syringe-filters-with-nylon-membrane-p-36371	LINK
13mm	SPECIFICATIONS

syringe filters for organic matrices-

Equipment

Syringe Filters, 13 mm PTFE	NAME
syringe filter	TYPE
Cytiva	BRAND
2400	SKU
https://www.cytivalifesciences.com/en/us/shop/lab-filtration/syringe-filters-non-sterile/wwptfe-non-sterile-syringe-filters/acrodisc-syringe-filters-with-universal-membranes-p-36635	LINK
13mm	SPECIFICATIONS

guard column holder-

Equipment

SecurityGuard ULTRA holder	NAME
Guard column holder	TYPE
Phenomenex	BRAND
AJ0-9000	SKU
https://www.phenomenex.com/part?partNo=AJ0-9000	LINK
2.1 to 4.6mm ID	SPECIFICATIONS

guard column-

Equipment	
UHPLC C18 guard cartridge	NAME
guard column	TYPE
Phenomenex	BRAND
AJ0-8782	SKU
https://www.phenomenex.com/part?partNo=AJ0-8782	LINK
2.1mm ID	SPECIFICATIONS

analytical column-

Equipment	
Kinetex C18	NAME
analytical separation column	TYPE
Phenomenex	BRAND
00D-4475-AN	SKU
https://www.phenomenex.com/products/kinetex-hplc-column/kinetex-c18	LINK
2.1 mm x 100 mm, 1.7 µm	SPECIFICATIONS

Instrumentation:

Equipment	
6470 Triple Quad LC/MS	NAME
LC-QQQ System	TYPE
Agilent Technologies	BRAND
G6470A	SKU

Equipment	
1290 Infinity UHPLC	NAME
Ultra-high performance liquid chromatography system	TYPE
Agilent Technologies	BRAND
1290 Infinity UHPLC	SKU
https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/analytical-hplc-systems	LINK

SAFETY WARNINGS

All chemicals used for this procedure are hazardous. Read the Safety Data Sheet (SDS) for all chemicals and follow all applicable chemical handling and waste disposal procedures. Manufacturer specific SDS information can be found by following the CAS numbers of compounds in 'Materials' list.

BEFORE START INSTRUCTIONS

All solvents and chemicals used are listed in the ‘Materials’ section. These are excluded from in-line references to maintain clarity and keep the steps concise.

Preparation of Standards

1

- By weight, create individual 2000 µg/mL stock solutions of all analytes listed below using ultrapure water (18.2MΩ·cm)(UPW) as a diluent, except for 6-aminohexanoic acid cyclic-dimer in which methanol is used:
- ε-Caprolactam
- 6-Aminohexanoic acid
- 6-Aminohexanoic acid dimer (6-(6-aminohexanamido)hexanoic acid)
- 6-Aminohexanoic acid trimer (6-(6-(6-aminohexanamido)hexanamido)hexanoic acid)

- 6-Aminohexanoic acid cyclic-dimer (18-diaza-29-diketocyclotetradecane)

2 Combine the stock solutions to make a 400 µg/mL mixed standard working solution (mSWS) in UPW.
1. This is accomplished by mixing equal volumes of the stock solutions of all 5 analytes
2. Prior to creating the calibration curve, perform an additional dilution of the mSWS. The final concentration will be 10 µg/mL. This can be prepared by adding 0.5 mL of the mSWS to 19.5 mL of UPW.

3 1. Using the mSWS at 10 µg/mL, create a calibration curve with a minimum of 5 points using UPW as a diluent.

Calibration curve preparation

Calibration level	Concentration (µg/mL) (ppm)	Volume of mSWS (µL)	Volume of UPW (µL)	Total volume (µL)
1	0.010	10 µL of level 5	990	1000
2	0.020	100 µL of level 3	900	1000
3	0.200	20	980	1000
4	0.500	50	950	1000
5	1.000	100	900	1000
6	2.000	200	800	1000
7	2.500	250	750	1000
8	5.000	500	500	1000
9	6.000	600	400	1000
10	7.000	700	300	1000

Table showing an example standard curve preparation. (click to enlarge)

Sample Preparation

4 Ensure sample matrix is compatible with instrumentation. The calibration range for this method is between 0.01 µg/mL and 7 µg/mL. All samples should be 0.2µm filtered prior to injection. Any samples expected to have analyte concentrations that fall outside of this range should be diluted appropriately.

Note: Analyte suppression was observed in high salt containing samples. As a result, a 5x dilution was performed using methanol to precipitate the salts. The samples were then 0.2µm filtered and analyzed.

UHPLC- MS/MS Analysis

5 Prepare an Agilent 1290 UHPLC system according to the following parameters for a total run time of 7.0 minutes:

Binary pump configuration	
Flow rate	0.5 mL / min
Maximum pressure	1200 bar
Mobile Phase A	0.1% formic acid in UPW (v/v)
Mobile Phase B	0.1% formic acid in methanol (v/v)

Gradient configuration		
Time (min)	Composition A (%)	Composition B (%)
1.00	99.00	1.00
4.00	50.00	50.00
5.00	20.00	80.00
6.00	99.00	1.00
7.00	99.00	1.00

Multisampler parameters	
Injection volume	0.5 µL
Draw speed	200 µL/min
Eject speed	200 µL/min
Wait time after draw	0 sec
Bottom sensing	Yes

Column compartment parameters	
Temperature	40 °C

Defined UHPLC parameters (click to enlarge)

6 Analyze samples using an Agilent 6470A triple quadrupole mass spectrometer equipped with dual Agilent jet stream electrospray ionization (AJS ESI) utilizing the source method parameters illustrated below.

Source parameters	
Gas flow	7 L/min
Nebulizer pressure	35 psi
Sheath gas temperature	350 °C
Sheath gas flow rate	11 L/min

Mass spectrometry parameters	*Negative*	*Positive*
Capillary voltage	3000 V	3000 V
Nozzle voltage	0 V	0 V

Defined MS/MS parameters (click to enlarge)

Below a table is provided with the optimized dynamic multiple reaction monitoring reactions (dMRM) as well as corresponding fragmentor voltages (V) and collision energies (CE) for the quantifying and qualifying transitions.

MRM transition table

Analyte name	Precursor ion	Ion	MRM quantifying transition	Collision energy (V)	Fragmentor (V)	MRM qualifying transition	Collision energy (V)
ε-caprolactam	114.1	[M-H]+	114.1 → 55.2	28	107	114.1 → 96.1	16
6-aminohexanoic acid (6-AHA)	132.1	[M-H]+	132.1 → 114.1	8	65	132.1 → 79.1	16
cyclic 6-aminohexanoic acid dimer (cyclic 6-AHA dimer)	227.2	[M-H]+	227.2 → 96.1	24	134	227.2 → 114.1	24
6-aminohexanoic acid dimer (6-AHA dimer)	245.2	[M-H]+	245.2 → 114.1	24	87	245.2 → 228.1	16
cyclic 6-aminohexanoic acid trimer (cyclic 6-AHA timer)	340.3	[M-H]+	340.3 → 114.1	36	N/A	N/A	N/A
6-aminohexanoic acid trimer (6-AHA trimer)	358.3	[M-H]+	358.3 → 114.1	36	124	358.3 → 245.1	24
6-aminohexanoic acid tetramer (6-AHA tetramer)	471.4	[M-H]+	471.4 → 114.1	48	N/A	N/A	N/A
6-aminohexanoic acid pentamer (6-AHA pentamer)	584.4	[M-H]+	584.4 → 114.1	72	N/A	N/A	N/A

dMRM analyte transitions (click to enlarge)

Note: Standards could not be sourced for 6-aminohexanoic acid cylic-trimer, 6-aminohexanoic acid tetramer, and 6-aminohexanoic acid pentamer. These were optimized from a sample that verified their presence by high resolution mass spectrometry ESI (HRMS-ESI).

Data Analysis and Quality Control

7 Data analysis completed using Agilent Quantitative Analysis for QQQ version 10.1

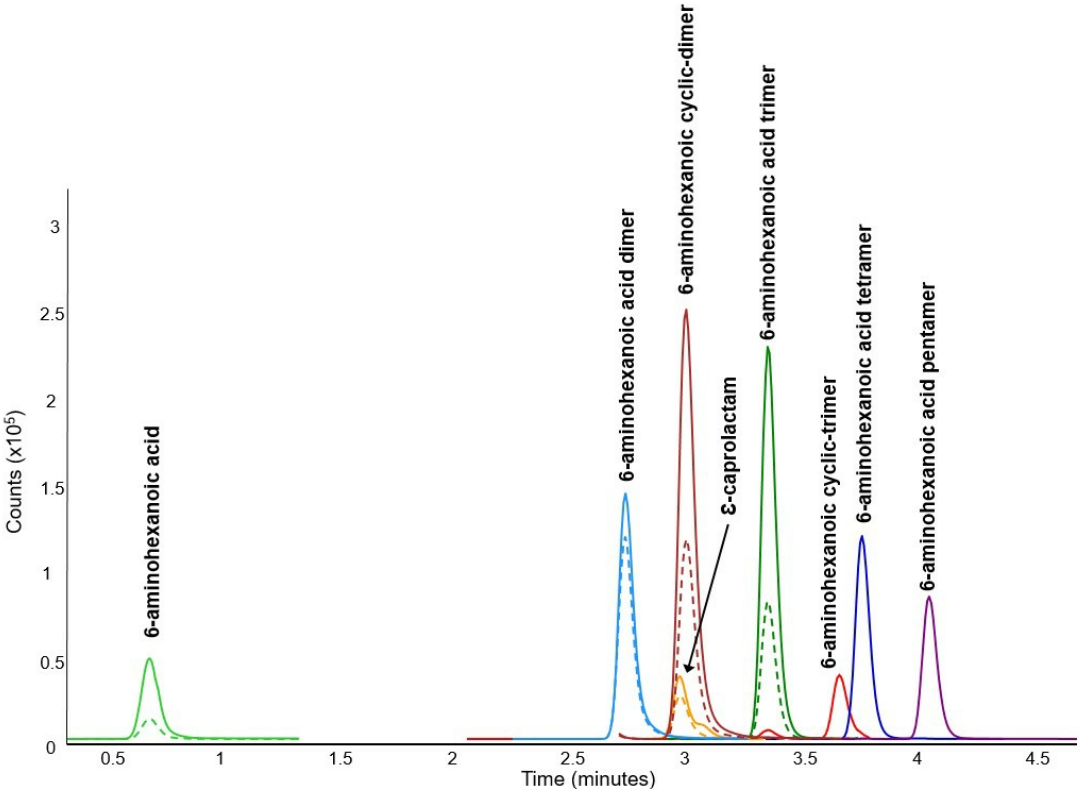
8 Quality Control

Several criteria are used to ensure instrument stability and reproducibility throughout the analysis.

- Calibration curves must have a correlation coefficient (r^2) of greater than or equal to 0.995 using a quadratic or linear fit.
- 6-Aminohexanoic acid cyclic-trimer is quantified using the calibration curve of 6-aminohexanoic acid cyclic-dimer
- 6-Aminohexanoic acid tetramer and 6-aminohexanoic acid pentamer are quantitated using the calibration curve of 6-aminohexanoic acid trimer.
- A calibration verification standard (CVS) is a standard from the calibration curve that is analyzed every 20 or fewer samples to check for instrument drift. For this analysis method, acceptable CVS recovery range is within +/- 15% of the expected amount.

Example Chromatogram

9



An example chromatogram of a sample containing deconstruction products of nylon-6. The solid line indicates quantifying MRM transition and dashed lines represent the qualifying MRM transistion (when available). (click to enlarge)