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Polyethylene terephthalic acid deconstruction product analysis by UHPLC-DAD

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We use this protocol and it's working

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Disclaimer

This protocol is for research purposes only.

Abstract

This analytical procedure outlines a rapid and accurate method for the quantification of three key PET deconstruction products, namely terephthalic acid (TPA), 2-hydroxyethyl terephthalic acid (MHET), and bis(2-hydroxyethyl) terephthalic acid (BHET), using an ultra high performance liquid chromatography with diode array detection (UHPLC-DAD) system. This method combines the advantages of UHPLC for improved resolution and separation efficiency with the sensitivity of DAD for accurate and reliable quantification.

Guidelines

This protocol utilizes an ultra high performance liquid chromatography diode array detector (HPLC-DAD) system manufactured by Agilent Technologies as referenced in 'Materials'. A similar UHPLC-DAD system can be utilized however, some parameter nomenclature may deviate depending on the manufacturer.

Materials

Standard Materials

- Terephthalic Acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**185361
- 2-Hydroxyethyl Terephthalic Acid **Toronto Research Chemicals Inc Catalog #**H942275
- Bis(2-Hydroxyethyl) Terephthalic Acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**465151

Standard Reagents

- Potassium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P5655
- Potassium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3786
- Tetrahydrofuran **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**401757

Mobile Phase Reagents

- Phosphoric Acid ACS 85 wt. % in Water **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**695017
- Methanol Optima **Fisher Scientific Catalog #** A454SK

Guard Column

Equipment

Zorbax Eclipse Plus C18	NAME
Guard Column	TYPE
Agilent	BRAND
821725-901	SKU
https://www.agilent.com/store/productDetail.jsp?catalogId=821725-901	LINK
2.1 × 5 mm 1.8 µm	SPECIFICATIONS

Analytical Column

Equipment

Zorbax Eclipse plus C18 Rapid Resolution HD	NAME
analytical separation column	TYPE
Agilent	BRAND
959757-902	SKU
https://www.agilent.com/store/en_US/Prod-959757-902/959757-902	LINK
2.1 × 50 mm, 1.8 µm	SPECIFICATIONS

Instrumentation:

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

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 Terephthalic acid (TPA) preparation

Prepare a buffer solution for the TPA standard

1. Prepare a 1M potassium phosphate dibasic (K_2HPO_4) buffer solution by weighing 87 g of K_2HPO_4 into 500 mL of 18.2MΩ·cm ultrapure water (UPW).
2. Prepare a 1M potassium phosphate monobasic (KH_2PO_4) buffer solution by weighing 68 g of KH_2PO_4 into 500 mL of UPW.
3. Prepare a 1M phosphate buffer solution by combining 94 mL of K_2HPO_4 (dibasic) buffer and 6 mL of KH_2PO_4 (monobasic) buffer.
4. Check that the pH of the buffer solution is 8.0 ± 0.1 pH units (7.9-8.1). Adjust pH with 10N NaOH or concentrated phosphoric acid if necessary.

Prepare the TPA standard

1. Weigh out dry TPA standard and create 1000 µg/mL TPA stock standard using the phosphate buffer solution created in the previous step as the stock standard diluent. Example: Weighed 9,800 µg (9.8 mg) of powdered TPA and add 9.8 mL of phosphate buffer solution.
2. Create a 250 µg/mL TPA working standard using the 1000 µg/mL TPA stock standard and UPW as the diluent. Example: Pipetted 2.5 mL of 1000 µg/mL TPA stock standard and add 7.5 mL of UPW.
3. Create the following calibration standards using the 250 µg/mL TPA standard working solution and UPW as the diluent.

Calibration Curve Preparation

Calibration Level	Concentration (µg/mL) (ppm)	µL of TPA Standard	µL of 18.2MΩ Water	Total Volume (µL)
1	1	100 of 10 µg/mL	900	1000
2	5	100 of 50 µg/mL	900	1000
3	10	100 of 100 µg/mL	900	1000
4	25	100 of 250 µg/mL	900	1000
5	50	200 of 250 µg/mL	800	1000
6	75	300 of 250 µg/mL	700	1000
7	100	400 of 250 µg/mL	600	1000
8	150	600 of 250 µg/mL	400	1000
9	250	1000 of 250 µg/mL	0	1000

Example calibration curve preparation for TPA (Click to enlarge)

2 2-Hydroxyethyl terephthalic acid (MHET) preparation

1. Prepare a 1:1 solution of UPW and tetrahydrofuran (THF)
2. Weigh out neat MHET standard and create a 1000 µg/mL MHET stock standard using the 1:1 solution of ultrapure water (18.2MΩ·cm) and THF created in the step 2.1. Example: weigh 8,700 µg (8.7 mg) of powdered MHET and added 8.7 mL of 1:1 UPW and THF.
3. Create a 250 µg/mL MHET working standard using the 1000 µg/mL MHET stock standard and the 1:1 solution of UPW and THF. Example: Pipetted 2.5 mL of 1000 µg/mL MHET stock standard and 7.5 mL of 1:1 UPW and THF solution.
4. Create the following calibration standards using the 250 µg/mL MHET standard working solution and UPW as the diluent.

Calibration Curve Preparation

Calibration Level	Concentration (µg/mL) (ppm)	µL of MHET Standard	µL of 18.2MΩ Water	Total Volume (µL)
1	1	100 of 10 µg/mL	900	1000
2	5	100 of 50 µg/mL	900	1000
3	10	100 of 100 µg/mL	900	1000
4	25	100 of 250 µg/mL	900	1000
5	50	200 of 250 µg/mL	800	1000
6	75	300 of 250 µg/mL	700	1000
7	100	400 of 250 µg/mL	600	1000
8	150	600 of 250 µg/mL	400	1000
9	250	1000 of 250 µg/mL	0	1000

Example calibration curve preparation for MHET (Click to enlarge)

3 Bis(2-hydroxyethyl) terephthalic acid (BHET) preparation

1. Weigh out neat BHET standard and create a 1000 µg/mL BHET stock standard using tetrahydrofuran (THF). Example: Weighed 9,500 µg (9.5 mg) of powdered BHET and added 9.5 mL of THF.
2. Create a 250 µg/mL BHET working standard using the 1000 µg/mL stock standard and THF. Example: Pipetted 2.5 mL of 1000 µg/mL stock standard and 7.5 mL of THF.
3. Create the following calibration standards using the 250 µg/mL BHET standard working solution and THF as the diluent.

Calibration Curve Preparation

Calibration Level	Concentration (µg/mL) (ppm)	µL of BHET Standard	µL of THF	Total Volume (µL)
1	1	100 of 10 µg/mL	900	1000
2	5	100 of 50 µg/mL	900	1000
3	10	100 of 100 µg/mL	900	1000
4	25	100 of 250 µg/mL	900	1000
5	50	200 of 250 µg/mL	800	1000
6	75	300 of 250 µg/mL	700	1000
7	100	400 of 250 µg/mL	600	1000
8	150	600 of 250 µg/mL	400	1000
9	250	1000 of 250 µg/mL	0	1000

Example calibration curve preparation for BHET (Click to enlarge)

Sample Preparation

4
- Samples must be filtered through a 0.2 µm or smaller filter prior to injection on the UHPLC
- Samples expected to be over the linear range of the instrument should be diluted to ensure accurate analysis and avoid carryover.

Mobile Phase Preparation

5 *Mobile Phase A:*
- a solution of 20 mM phosphoric acid in UPW using the ratio of 1.34 mL of 85% phosphoric acid per 1 L of UPW. Ensure sufficient volume for analysis of all samples and standards.

Mobile Phase B:
- Methanol

UHPLC-DAD Parameters

6

Binary pump configuration	
Flow rate	0.7 mL / min
Maximum pressure	1300 bar
Mobile phase A	20mM phosphoric acid (v/v)
Mobile phase B	methanol (v/v)

Gradient configuration		
Time (min)	Composition A (%)	Composition B (%)
0.00	80.00	20.00
2.00	35.00	65.00
2.01	80.00	20.00
3.00	80.00	20.00

Column compartment parameters	
Temperature	40 °C

Multisampler parameters	
Injection volume	0.25 µL
Draw speed	100 µL/min
Eject speed	400 µL/min
Wait time after draw	2 sec
Bottom sensing	enabled

Diode array configuration	
Wavelength:bandwidth (reference)	240:4 (360:100)
Peakwidth	>0.013 min (20Hz)
Spectra	store all

Analytical Quality Control

7 *Calibrations*

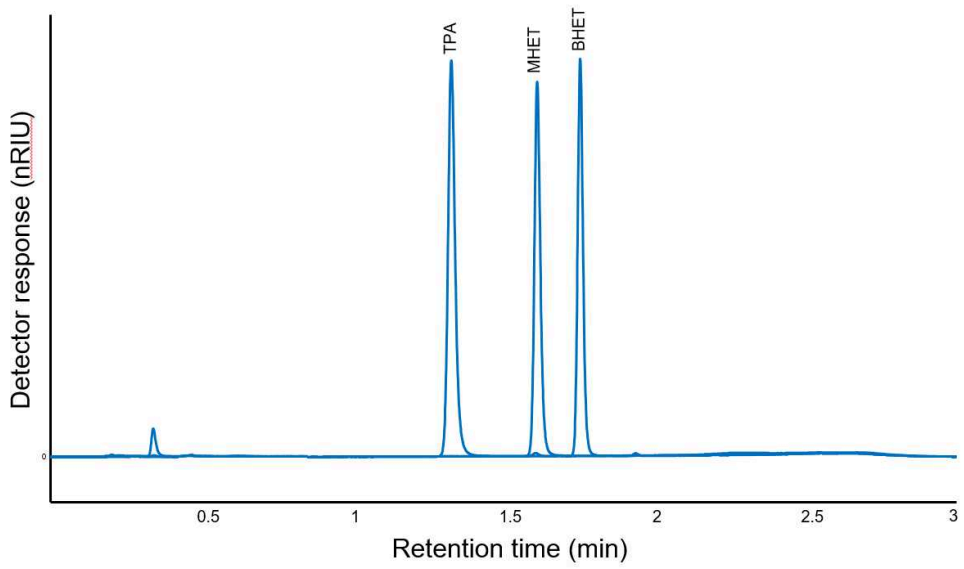
- All compounds must have a correlation coefficient (r^2) of greater than or equal to 0.995 using a linear fit. It is recommended to weight the calibration with a 1/x weighting.

Calibration verification standards

- A calibration verification standard (CVS) is a standard from the calibration that is re-analyzed every 20 or fewer samples to ensure instrument drift remains within the determined acceptable criteria. Acceptable recoveries for this analysis are ±10% of the expected value. All reported data was bracketed by acceptable CVS recoveries. Acceptance criteria may differ between instruments and should be determined experimentally.

Example Chromatogram

8



Example chromatogram

Protocol references

Erickson, E., Gado, J.E., Avilán, L. *et al.* Sourcing thermotolerant poly(ethylene terephthalate) hydrolase scaffolds from natural diversity. *Nat Commun* **13**, 7850 (2022). https://doi.org/10.1038/s41467-022-35237-x