HPLC Analysis of Degradation Products

To analyze the enzymatic degradation products, the reaction mixture was centrifuged at 13,000 × g for 15 min, and the supernatant was collected for subsequent analysis. High-performance liquid chromatography (HPLC) was performed using a Waters e2695 system equipped with a 2489 UV/Vis detector. Separation was carried out on an Eclipse Plus-C18 column (5 μm, 4.6 × 250 mm). Prior to analysis, the column was conditioned with pure methanol for 2 h at a flow rate of 0.5 mL/min.

After activation, the column was equilibrated with mobile phase A (0.1% trifluoroacetic acid in water) and mobile phase B (HPLC-grade methanol) at a ratio of 95% A to 5% B until a stable baseline was achieved. The injection volume was set to 10 μL, and the following gradient elution program was applied: from 0 to 5 min, solvent B was increased from 5% to 40%; from 5 to 20 min, solvent B was increased from 40% to 45%. The analysis was performed at 25 °C with a flow rate of 0.8 mL/min.

Detection was carried out at 260 nm to quantify bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate (MHET), and terephthalic acid (TPA). Data acquisition and processing were performed using the Waters Empower software to ensure accurate quantification of the target compounds.