

# Quantification of the monomer compositions of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxyvalerate) by alkaline hydrolysis and using high pressure liquid chromatography

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**Abstract:** With the growing interest in bioplastics, there is an urgent need to develop rapid analysis methods linked to production technology development. This study focused on the production of a commercially non-available homopolymer, poly(3-hydroxyvalerate) P(3HV), and commercially available copolymer, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)), through fermentation using two different bacterial strains. The bacteria, *Chromobacterium violaceum* and *Bacillus* sp. CYR1, were used to produce P(3HV) and P(3HB-co-3HV), respectively. The bacteria *Bacillus* sp. CYR1 produced 415 mg/L of P(3HB-co-3HV) when incubated with acetic acid and valeric acid as the carbon sources, whereas the bacteria *C. violaceum* produced 0.198 g of P(3HV)/g dry biomass when incubated with sodium valerate as the carbon sources. Additionally, we developed a fast, simple, and inexpensive method to quantify the P(3HV) and P(3HB-co-3HV) using high performance liquid chromatography (HPLC). As alkaline decomposition of P(3HB-co-3HV) releases 2-butenic acid (2BE) and 2-pentenoic acid (2PE), we were able to determine the concentration using HPLC. Moreover, calibration curves were prepared using standard 2BE and 2PE, sample 2BE and 2PE which are produced by alkaline decomposition of poly(3-hydroxybutyrate) and P(3HV), respectively. Finally, the HPLC results obtained from our new method were compared using gas chromatography (GC) analysis.

**Keywords:** chromatography; crotonic acid; homopolymer; copolymer; polyhydroxyvalerate

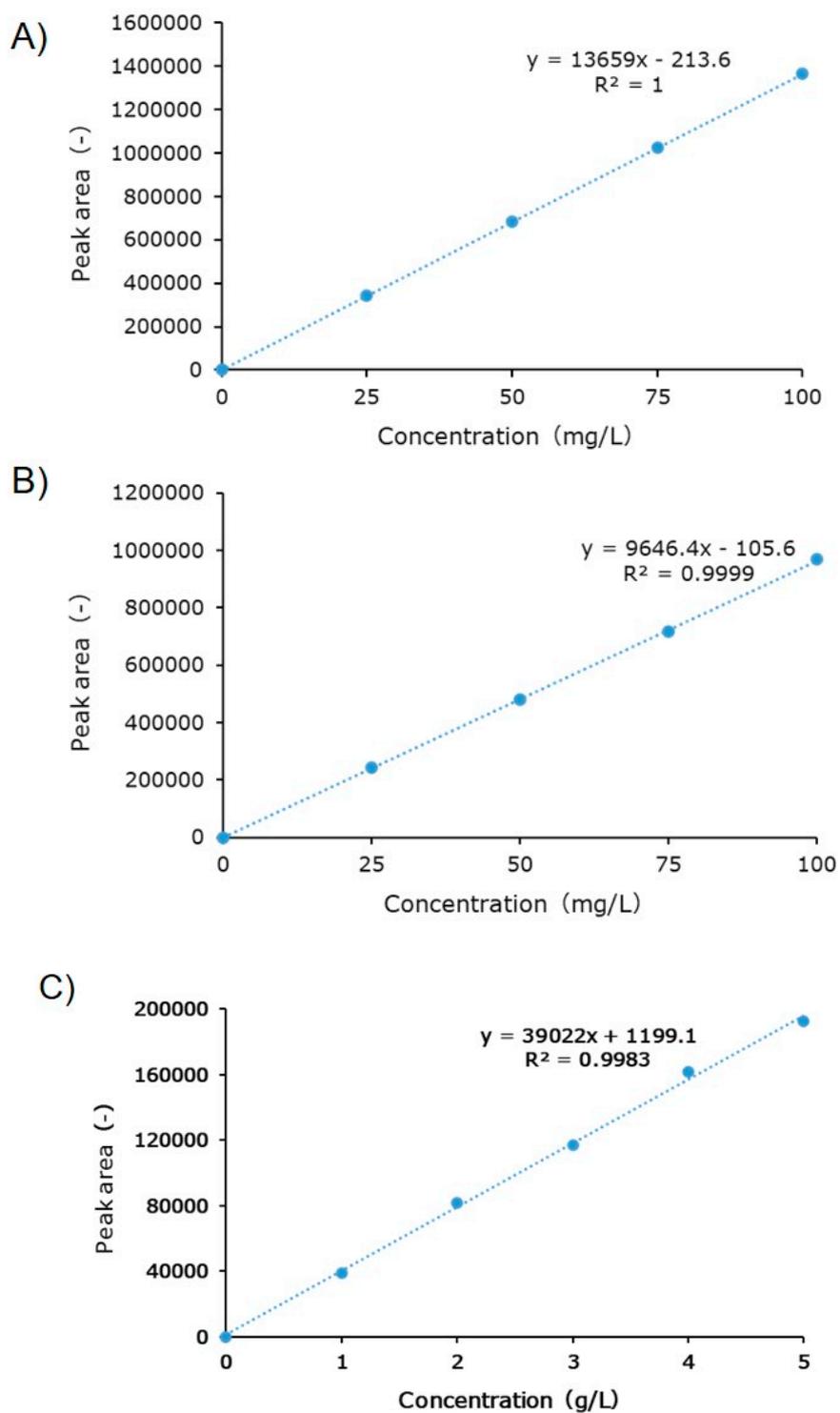


Figure S1. Calibration curves of (A) 2BE, (B) 2PE, and (C) 3HB. The 2BE, 2PE, and 3HB were purchased from Kanto Chemical (Japan), Tokyo Chemical Industry (Japan), and Sigma-Aldrich, respectively.

The absorption sensitivities (as the peak area/concentration) for 2BE ( $1.36 \times 10^4$ ), 2PE ( $9.69 \times 10^3$ ), and 3HB ( $3.9 \times 10$ ) were determined. It was found that 2-alkenoic acid could be detected with higher sensitivity than 3HB because it is an unsaturated fatty acid with multiple bonds in its structure, which leads to strong UV absorption. By comparing the absorption sensitivities of 2BE and 2PE, it was found that 2BE can be detected with 1.4-fold better sensitivity than 2PE.

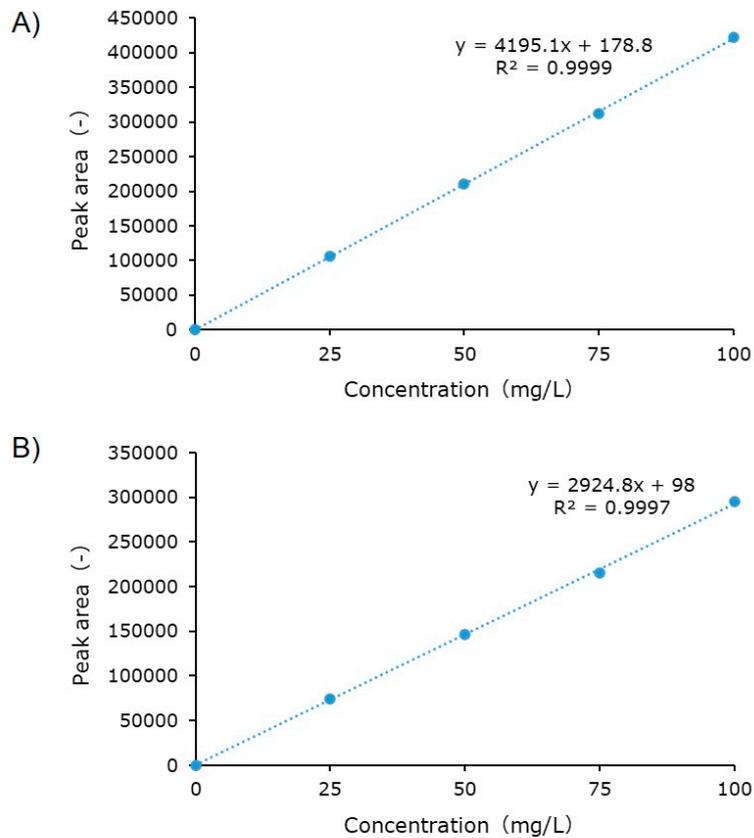


Figure S2. Calibration curves of (A) 2BE, and (B) 2PE. 2BE and 2PE were generated by the pretreatment of P(3HB) and P(3HV) using alkaline decomposition. P(3HB) was purchased from Sigma-Aldrich. Since there is no standard sample for P(3HV), it was produced in this study from *Chromobacterium violaceum*. From the ratio of the slopes of the calibration curve, the production ratios were determined as  $\alpha = 3.26$  and  $\beta = 3.30$ . These results indicate that short-chain-length PHA (scl-PHA) can be quantified easily and with high sensitivity by measuring the 2BE and 2PE contents of actual samples using the method proposed here.