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Improved Analytical Precision of 1,4-Dihydroxy-2-naphthoic Acid by High Performance Liquid Chromatography Using Dithiothreitol as Mobile Phase Additive

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1,4-dihydroxyl-2-naphthoic acid (DHNA) is a bifidogenic growth stimulator from *Propionibacterium freudenreichii*. According to a method described earlier, the peak area of DHNA measured by a high-performance liquid chromatography (HPLC) gradually increases with repeated analyses of the same sample. We hypothesized that the oxidizability of DHNA was the cause of poor precision. Therefore, we attempted to develop an improved method using dl-dithiothreitol (DTT) as a mobile phase additive. A DHNA standard solution (5 µg/ml) was analyzed five times in a row by either the original or the improved method. The relative standard deviation (R.S.D.) of the peak area was 37.0% and 1.6%, respectively. The linearity of the improved method was confirmed in the range of 0.25-10 µg/ml ($R^2 = 0.9998$). These data indicate that the addition of DTT to the mobile phase improves precision of the analysis of DHNA by HPLC.

Keywords: [1,4-dihydroxyl-2-naphthoic acid \(DHNA\)](#), [HPLC analysis](#), [dithiothreitol \(DTT\)](#), [mobile phase additive](#)

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