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**Separation of Photosynthetic Pigments by  
High-Performance Liquid Chromatography: Comparison of Column  
Performance, Mobile Phase, and Temperature**

**Indriatmoko<sup>a</sup>, Yuzo Shioi<sup>a</sup>, Tatas Hardo Panintingjati Brotosudarmo<sup>a</sup>,  
Lecenawaty Limantara<sup>a\*</sup>**

<sup>a</sup>Ma Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, Malang 65151, East Java, Indonesia

**Abstract**  
High-performance liquid chromatography (HPLC) has been commonly used as method of separating and identifying photosynthetic pigments such as chlorophylls and carotenoids because of such advantages as speed, high resolution and sensitivity. In this technique, high separation relies largely on the type of column material. This study compared the efficiency of five reverse-phase columns, C<sub>8</sub>, C<sub>18</sub>, C<sub>18</sub> monolithic,  $\pi$ -NAP, and cholesterol, for separation of photosynthetic pigments at several fixed conditions of mobile phase and temperature. This investigation also analysed the parameters of  $\Delta k'$  and  $k'$  ratio for selected pigments and resolution for structural isomers, such as *o*- and *p*-carotenes. Among above columns tested, cholesterol column is suitable for separation of pigments not only for a broad range of polarity, but also for hydrophobic pigments in a simple mobile phase. This finding can help in the selection of column and HPLC parameters in separating photosynthetic pigments.  
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**Keywords:** Cholesterol bonded, HPLC column, monolithic packing, particulate packing, photosynthetic pigments, reverse phase.

\*Corresponding author. Tel: +62 813 2636 0303, Fax: +62 341 550 175  
E-mail address: [lecenawaty.limantara@machung.ac.id](mailto:lecenawaty.limantara@machung.ac.id)

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