PURIFICATION OF HbA_2 AND HbE BY MEDIUM PRESSURE LIQUID CHROMATOGRAPHY (MPLC) WITH SPECIAL GRADIENT PATTERNS

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ABSTRACT

HbA_2 is a minor hemoglobin in human blood accounting for approximately 2.5% in normal. HbE is an abnormal hemoglobin constituting 25-30% in typical carriers. In general, MPLC separation of Hbs A_2 or E takes about 7 hours under ordinary gradient pattern. This study aimed to develop the new protocol for purification of Hbs A_2 and E using the MPLC system; i.e. to reduce preparation duration. This technique was based on an anion-exchange chromatography run in the AKTA *prime* system. Seven mL DEAE-sepharose FF packed in 10 cm x 10 mm-column was utilized as stationary phase and Tris-HCl-KCN buffer as mobile phase. One mL hemolysate mixed with 4 mL DW was loaded into the column. Flow rate of mobile phase was set at 1.0 mL/min and back pressure at 0.4 MPa. It was found that when Buffer B (pH 6.0) started at 21% and increased at the rate of 1%/6 min, HbA_2 or HbE was eluted at 25 min to 40 min and cleary separated from HbA. It was concluded this new protocol was quick and suitable for preparating HbA_2 or HbE for use as standard hemoglobins or as immunogen for antibody production.

KEYWORDS: HEMOGLOBIN, HEMOGLOBIN PURIICATION, MEDIUM PRESSURE LIQUID CHROMATOGRAPHY, ANION EXCHANGE CHROMATOGRAPHY, AKTA*prime*

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