Conditions of slightest amount dNMPs quantification by micellarelectrokinetic chromatography

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Capillary electrophoresis (CE) has the advantages of achieving good separation and requiring small volume of biomolecules. A micellarelectrokinetic chromatography (MEKC), one of the CE mode using a cationic surfactant to reverse electroosmotic force (EOF), is appropriate to separate and quantify 2’-deoxyribonucleoside 5’-monophosphates (dNMPs). In MEKC mode, reversal of the direction of EOF is achieved by addition of a cationic surfactant such as cetyltrimethylammoniumbromide (CTAB), functions the pseudostationary phase and cationic capillary wall modifier for EOF reversal. There are several important factors for dNMPs separation; CTAB concentration, pH and the salt concentration of the background electrolyte (BGE). Even though separation conditions are well established, the detailed stacking conditions for small amount of dNMPs quantification are not well known. Sample stacking in MEKC is an on-line sample concentration technique to increase the low detection sensitivity. Because of low injection volume (nL) and short pathlength, it is hard to detect and quantify the low concentration biomolecules compare with HPLC. In this study, we changed three stacking conditions - stacking time, CAPSO concentration in stacking buffer, glycerol content in sample buffer - to improve sensitivity of dNMPs detection using UV photometric detector which is the most cost-effective and probably available with all classical CE systems. Because the results showed that the longer stacking time was required for quantitation of low concentration of dNMPs, 60’ stacking time was applied for quantitation of dNMPs on the assumption that there were no analogue contaminants in the sample. Also, the variation of CAPSO and glycerol concentration did not affect separation of dNMPs and integration of peak’s area.

Biography

Kyong Hwa Oh received her bachelor’s degree from Chungnam National University in 2007. She is currently a master’s degree course at the University of Science and Technology. Her major is bioanalysis science. She studies about quantification of biomolecules (DNA and proteins) and method development or reference material certification. She has published papers in JOURNAL OF CHROMATOGRAPHY as a co-author in 2012.

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