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High performance and ultra-high performance liquid chromatography for determination of organic acids - intermediates of branched-chain amino acids biosynthesis in *Escherichia coli* strains

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Recent advances in the development of branched-chain amino acids (BCAA) production strains is mainly based on using of system metabolic engineering strategies. Such approach needs thorough understanding of BCAA biosynthetic pathways and its regulations. In particular, the qualitative and quantitative evaluation of organic acids (OA), which are intermediates of BCAA biosynthesis, may help to clarify the key points of this process. Currently, different analytical methods are used for OA determination, including gas chromatography (GC), HPLC and mass spectrometry (MS). From other hand, the UHPLC approach is able to provide the analysis of numeral culture fluid (CF) samples with high sensitivity and high speed. The assessment of possibility to apply UHPLC and HPLC approaches for OA determination in CF of *E. coli* strains is the goal of this work. The following OA were analyzed: ketoisovalerate (KIV), 2-isopropylmalate (2-IPM), 3-isopropylmalate (3-IPM), ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), ketoisobutyrate (KIB), ketoglutarate (KG). For each method, the capacity factor (K), number of theoretical plates (N), asymmetry of the peak (Fasy), relative standard deviation by area (RSDPA), relative standard deviation of migration retention time (RSDRT) were calculated. It was shown that method UHPLC has lower qualitative detection limit, lower Fasy, RSDPA, and RSDRT. The time for analysis of one sample was much lower in case of UHPLC (5 min) in comparison with those for HPLC (10 min). Quantitative analysis of OA was performed in CF samples of *E. coli* strains. Two intermediates: KIV (1.53 mg/l from UHPLC and 1.87 mg/l from HPLC) and 2-IPM (2.07 mg/l from UHPLC and 2.37 mg/l from HPLC) were detected in analyzed samples. It was shown that the method UHPLC for determination of OA in CF of *E. coli* strains has a number of advantages, higher sensitivity and less analysis time, compared with HPLC.

Biography

Elizaveta Fedorova completed her Graduation from Russian State University named after A. N. Kosygin in 2006. She has been employed as an Analytical Chemist/ Engineer at Ajinomoto-Genetika Research Institute in 2007 and as Junior Research Associate since 2013. Her research interests include the development of UHPLC methods for analysis of organic acids in cultural fluids. She is the author of original research papers published in international journals.

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