Simultaneous analysis of the non-canonical amino acids norleucine and norvaline in biopharmaceutical-related fermentation processes by a new ultra-high performance liquid chromatography approach.

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Details

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Abstract

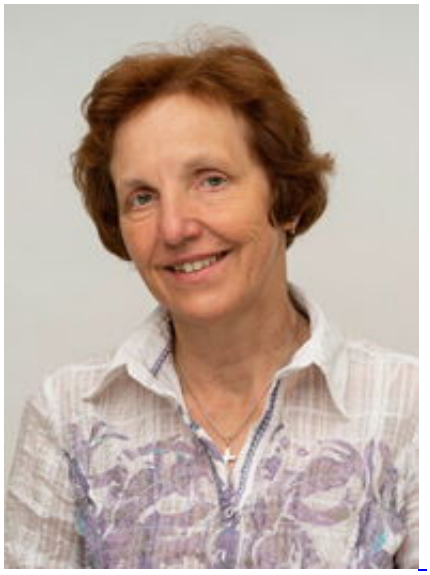
In this study, a precise and reliable ultra-high performance liquid chromatography (UHPLC) method for the simultaneous determination of non-canonical (norvaline and norleucine) and standard amino acids (aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine) in biopharmaceutical-related fermentation processes was established. After pre-column derivatization with ortho-phthaldialdehyde and 2-mercaptoethanol, the derivatives were separated on a sub-2 µm particle

C18 reverse-phase column. Identification and quantification of amino acids were carried out by fluorescence detection. To test method feasibility on standard HPLC instruments, the assay was properly transferred to a core-shell particle C18 reverse-phase column. The limits of detection showed excellent sensitivity by values from 0.06 to 0.17 pmol per injection and limits of quantification between 0.19 and 0.89 pmol. In the present study, the newly established UHPLC method was applied to a recombinant antibody Escherichia coli fermentation process for the analysis of total free amino acids. We were able to specifically detect and quantify the unfavorable amino acids in such complex samples. Since we observed trace amounts of norvaline and norleucine during all fermentation phases, an obligatory process monitoring should be considered to improve quality of recombinant protein drugs in future.

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