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HPLC Determination of L-valine, L-leucine, and L-Isoleicin Using Pre-column Derivatization by Di-tret-butyl-dicarbonate

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Cleaning equipment in the production of medicines is an important requirement of good manufacturing practice. On the same process equipment, as a rule, a number of different drugs are produced, which can lead to their cross contamination, for the prevention of which it is necessary to perform effective cleaning of equipment with validation of the procedures applied for each unit of equipment.

For the first time, methods for determining some amino acids (AA, L-valine, L-leucine and L-isoleucine) and their trace amounts in washings for purification of pharmaceutical equipment by reversed-phase high performance liquid chromatography with UV detection have been proposed for dietary supplementation. For pre-columnar derivatization, the reagent - di-tert-butyl dicarbonate, widely used in organic synthesis for protecting amino groups, has been used.

Chromatographic conditions and sample preparation procedures have been optimized. The developed methods for determining the AA are validated according to the following parameters: specificity, linearity, accuracy, detection limit.

The calibration curves are linear over the concentration range of L-leucine 3.0 - 300.0 μg/ml, L-isoleucine and L-valine 1.5 - 150.0 μg/ml. The detection limits of L-leucine, L-valine and L-isoleucine are 3.57 μg/ml, 1.61 μg/ml and 1.20 μg/ml, respectively. The degree of extraction of amino acids from the surface of pharmaceutical equipment is more than 90%.

Keywords: high-performance liquid chromatography, pre-column derivatization, amino acids, di-tert-butyl pyrocarbonate