OPTIMIZATION OF LIQUID CHROMATOGRAPHIC CONDITIONS TO SEPARATE AMINO ACIDS WITH 1-FLUORO-2,4-DINITROBENZENE PRECOLUMN DERIVATIZATION

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The article describes the optimization stages of liquid chromatographic separation parameters for amino acids (arginine, glutamine, serine, asparagine, threonine, glycine, alanine, proline, methionine, tryptophan, ornithine, lysine) with 1-fluoro-2,4-dinitrobenzene pre-column derivatization.

Separation was performed by Alliance 2690 separation module with Waters 996 photodiode array detector, equipped with reversed-phase chromatographic column Luna C18 (2) 250 × 4,6mm, 5 μm particle size column (Phenomenex, USA) at 25º C. For mobile phase acetonitile and 0.1 M solution of NaH_2PO_4 with pH 2.0 were used.

Flow rate of mobile phase was 1.0 ml /min. Injection volume was 10 μl and detection was carried at 350 nm. Isocratic and gradient schemes of elution have been investigated.

Changes in the quantitative parameters of chromatographic peaks are indicated for different conditions of separation of derivatized amino acids. The best peak (asymmetry, width and the number of theoretical plates) and separation parameters are registered when gradient elution was used. This type of elution enabled us to separate eleven derivatized amino acids in mixture.

Some validation characteristics are given, such as repeatability (RSD,%; 0,08–0,23), linearity (R^2=0,9990–0,9998), linearity range (1–15 μg/ml) and quantification limit (0,03–0,86 μg/ml).

The parameters of the peaks and the studied validation characteristics do not go beyond the limits recommended by the State Pharmacopoeia of Ukraine (SPU), which will allow further validation of the method for the quantitative determination of amino acids in different matrices: injectable and oral solutions, feeds and premixes.

Keywords: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, AMINO ACIDS, DERIVATIZATION, 1-FLUORO-2,4-DINITROBENZENE.