Abstract

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Title of thesis: HPLC evaluation of L-tryptophan and its metabolites in biological material

The purpose of this thesis was to develop optimized conditions for determination of L-tryptophan and its metabolites (L-kynurenine, kynurenic acid, serotonin, 5-hydroxyindole-3-acetic acid, melatonin) using high performance liquid chromatography.

Separation was achieved by a silica gel column Kinetex EVO C18 (100A, 150 × 3 mm, 5 μm) with guard column OPTI-GUARD 1 mm C18 using spectrophotometric and fluorimetric detection. Initial parameters of detection mentioned in the method were for kynurenine (absorbance at 369 nm, 227 nm and fluorescence detection Ex: 369 Em: 475). Detection and elution parameters of the method were further optimized for subsequently added analysed substances on the basis of their individual UV and fluorescence spectra.

Different types of mobile phase, different pH of buffer were examined. The finally mobile phase consisted of two components:

- mobile phase A: water + acetate buffer 0,1 M; pH 4,5; methanol in a ratio 97:3
- mobile phase B: methanol.

The separation was performed by gradient elution. The flow rate was 0,5 ml/min. The column temperature was set at 30 °C. The injection volume was 100 μl. Total runtime was 30 min.

HPLC analysis was validated according to FDA guidelines. Vanillin was used as the internal standard. Selectivity, stability, linearity, accuracy, precision – repeatability, and robustness were measured validation parameters and all found values were within acceptable ranges.

Keywords: L-tryptophan, L-kynurenine, kynurenic acid, serotonin, 5-hydroxyindole-3-acetic acid, melatonin, HPLC