Abstract

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Title of thesis: Development of HPLC method for evaluation of tryptophan metabolites

The essential amino acid tryptophan is a precursor of several important bioactive compounds. In recent years it was found out that tryptophan metabolites are involved in the pathogenesis of many diseases, including neurodegenerative and psychiatric disorders, autoimmune diseases, or cancer. Therefore, the analytical methods for simultaneous determination of tryptophan and its metabolites are highly required.

The purpose of this diploma thesis was to develop an optimal HPLC method for determination of tryptophan and its metabolites (kynurenine, kynurenic acid, serotonin and 5-hydroxyindole-3-acetic acid) and to consider all the possible options of determination of quinolinic acid and melatonin.

The chromatographic separation was carried out by Supelco Ascentis Express F5 column, 2.7 µm particle size, 15 cm × 3 mm, using spectrophotometric and fluorescence detection. Parameters of detection were for kynurenine UV detection 369 nm, 254 nm, fluorescence detection Ex/Em: 369/475; for kynurenic acid UV detection 244 nm; for tryptophan UV detection 300 nm, fluorescence detection Ex/Em: 280/334; for serotonin UV detection 280 nm, fluorescence detection Ex/Em: 280/334, for 5-hydroxyindole-3-acetic acid UV detection 276 nm, fluorescence detection Ex/Em: 276/333.

Various types of mobile phases were examined. The final mobile phase consisted of water + acetate buffer 0.1M pH 3.5/acetonitrile in a ratio 92/8. The separation was performed by isocratic elution. The flow rate was determined at 0.5 ml/min and the column temperature was set to 30 °C. The injection volume was 20 µl and total routine took 10 min. Furthermore, the method was validated according to FDA guidelines and all the validated parameters were within acceptable ranges.

Key words: tryptophan, kynurenine, kynurenic acid, serotonin, 5-hydroxyindole-3-acetic acid, quinolinic acid, melatonin, HPLC