

Abstract

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Title of thesis: Development and Validation of HPLC Method for Determination of 3-Hydroxykynurenine in Biological Material

3-Hydroxykynurenine is one of the metabolites of tryptophan and is studied especially for its oxidative modulatory activity, which provides the ability to protect cells from oxidative stress. It is formed from kynurenine by catalysis of the enzyme kynurenine monooxygenase, whose activity is monitored at the Department of Pharmacology and Toxicology. It is necessary to quantify 3-hydroxykynurenine in a biological matrix for its study.

The aim of this diploma thesis was to develop an HPLC method for the determination of 3-hydroxykynurenine in biological material using high performance liquid chromatography and subsequent method validation.

During development, several mobile phases with different pH values were tested. The resulting mobile phase consisted of 100 mM formic acid (pH = 2.25) and methanol (95:5). Different types of stationary phases were also tested, from which a YMC TRIART PFP column with dimensions of 15 × 3 mm and a particle size of 5 μm was finally chosen. A UV-VIS detector was selected to detect 3-hydroxykynurenine, on which a wavelength of 370 nm was set. The separation temperature was also optimized to a final value of 35 °C. Isocratic elution was used during the analysis, and the mobile phase flow rate was set at 0.3 ml/min. The injection volume was set at 10 μl and the total analysis time was 14 minutes.

The selected validation parameters were as follows: linearity, accuracy, precision, limit of quantification, selectivity, stability, carry-over and recovery. All validation parameters corresponded to acceptable ranges except for the stability parameter, which showed that the 3-hydroxykynurenine is relatively unstable, and its solutions should always be prepared at the time of use.

Keywords: 3-hydroxykynurenine, tryptophan, HPLC