Abstract

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Title of diploma thesis: Isolation of tryptophan metabolites from biological material

The topic and aim of this diploma thesis was to develop the most effective sample preparation before analysis for isolation of L-tryptophan and its metabolites (serotonin, melatonin, 5-hydroxyindole-3-acetic acid, L-kynurenine, kynurenic acid) from biological material, in this case from rabbit plasma.

Basic methods of sample preparation prior to analysis, namely liquid-liquid extraction (LLE), solid phase extraction (SPE) and deproteinization, were successively tested, with the latter yielding the highest yield.

Isolation was performed by mixing a 2.5 µl stock solution of all substances at a concentration of 10 µg/ml and 122.5 µl rabbit plasma. Deproteinization was performed by adding 500 µl of methanol, shaking and centrifuging at 9000 rpm for 5 minutes. Subsequently, 400 µl of supernatant was collected and analyzed by high performance liquid chromatography (HPLC).

The method developed in the diploma thesis "HPLC evaluation of L-tryptophan and its metabolites in biological material" (Kateřina Málková, 2019) was used for the chromatographic analysis. It was performed on a Kinetex EVO C18 silica gel column (100 A, 150 x 3 mm, 5 µm) with an OPTI-GUARD 1 mm C18 precolumn at 30 ° C with a flow rate of 0.5 ml / min. Mobile phase A was an acetate buffer at pH 4.5 with methanol in a ratio of 97:3. Mobile phase B was methanol. The optimal sample injection volume was 10 - 15 µl. The separation was performed by gradient elution and the analysis time was 30 minutes. The detectors used were spectrophotometric and fluorimetric.

The method was validated according to the EMA guideline. An external standard method was used to determine the substances. The validation parameters evaluated were accuracy, precision, selectivity, linearity, sensitivity. All values found were within a reasonable range.