ABSTRACT

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Title of diploma thesis: Determination of kynurenine and tryptophan in dried urine spot for clinical research and practice

The dried urine spot (DUS) is a modern sample preparation technique that requires only minimal amounts while simplifying handling and storage compared to conventional techniques. DUS also has a high potential for use because it provides improved stability of analytes in biological matrices. The subject of the research itself was the substances kynurenine and tryptophan. In clinical practice, these two substances are determined in biological matrices due to their connection with many biochemical processes and pathological conditions.

The aim of this diploma thesis was to develop and optimize the extraction method and also to perform an appropriate validation of the analytical method, which will subsequently apply to real samples. Samples prepared by the DUS method were extracted using 150 μL of a solvent containing 65% methanol with 0,2% formic acid and 35% 5 mM ammonium formate in water. These two solutions are also components of the mobile phase of the detection system. The extraction procedure further includes 5 min shaking, 5 min centrifugation and also filtration through a filter plate to remove any impurities. The internal standard L-tryptophan-D_5 was used for quantification. A chromatographic method combined with an analyzer with mass detection (UHPLC- MS/MS) was used for detection. This combination is widely used in the determination of trace amounts of substances in biological matrices.

Key words: kynurenine, tryptophan, dried urine spot, UHPLC-MS/MS