# ABSTRACT

**Charles University in Prague, Faculty of Pharmacy in Hradec Králové**

**Department of Analytical Chemistry**

**Candidate:** Kateřina Plachká

**Supervisor:** doc. PharmDr. Lucie Nováková, Ph.D.

**Title of Diploma Thesis:** Development and validation of methods for the determination of agomelatine using UHPSFC and UHPLC

Agomelatine is one of the newest antidepressants. Due to a different mechanism of action it offers a completely new approach in the treatment of depressive disorders. Two chromatographic methods for determination of agomelatine and its impurities were developed. The separations on UHPSFC system were accomplished using stationary phase based on BEH 2-EP and gradient elution using $CO_2$ and MeOH containing 20mM ammonium formate and 5 % of water. The gradient was run from 5 - 30 % of organic modifier in 3 min. The UHPLC separations were performed on stationary phase BEH Shield RP18. The mixture of acetonitrile and methanol in ratio 1:1 and 10mM ammonium acetate buffer pH 9.5 were used as mobile phase. Both developed methods were properly validated in terms of linearity, sensitivity (LOD, LOQ), accuracy and precision according to ICH guidelines. The UHPSFC method was linear in the range 0.25-70 µg/ml for all analytes with method accuracy ≥ 97.4 % and ≥ 100.2 % and method precision RSD ≤ 2.4 and ≤ 0.8 for impurities and API, respectively. The UHPLC method was linear in the range 0.1-10 µg/ml for all analytes except three impurities for which the linear range was larger 0.1- 25 µg/ml. Method accuracy ≥ 95.7 % and ≥ 95.2 % and method precision RSD ≤ 2.6 and ≤ 1.5 were found out for impurities and API, respectively.

The measurement of tablet samples was performed and the methods were compared in the selected parameters. In conclusion, both methods were appropriate for the determination of agomelatine and its impurities in pharmaceutical quality control, although the UHPSFC method was found as slightly more convenient. The advantages of newly developed UHPSFC-UV method are its environmental friendliness due to mobile phase used, a good resolution, selectivity and high speed of analysis (total time of separation is 4.1 min).

**Keywords:** agomelatine; method development; UHPSFC; UHPLC; optimization; quality control