Abstract

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry Candidate: Bc. Moldrzyková Karolína

Supervisor: prof. PharmDr. Lucie Nováková, Ph.D.

Title of the diploma thesis: The Development of a UHPLC-MS/MS method for the analysis of a selected group of neurotransmitters

The aim of this thesis was to develop and optimize a method for the separation and identification of a group of 10 selected neurotransmitters using ultra-high-performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS).

In the first step, a MS scan was performed to gain *m/z* of precursor ions, the retention time of standards was monitored. The formation of $[M+H]^+$ and $[M-H]^-$ ions was also verified. The most intense fragments of the individual analytes, identified from product ion scans, were selected for SRM. Optimization of the collision energy and the ion source setting was conducted. This initial measurement was carried out on an ACQUITY UPLC BEH C18 column. In stationary phase screening were tested, three reversed-phase columns (ACQUITY UPLC BEH C18, Kinetex F5, ACQUITY UPLC BEH Shield RP C18) and six HILIC columns (Atlantis Premier BEH Z-HILIC, Luna NH2, ACQUITY UPLC BEH Amide, Syncronis HILIC, ACQUITY UPLC BEH HILIC, CORTECS UPLC HILIC). Formic acid in water, ammonium acetate (pH 4, 6, and 9), ammonium formate (pH 3) were tested, as the aqueous component of the mobile phase while acetonitrile and acidified acetonitrile were tested as the organic components.

A short-term stability study was conducted over 24 hours. The change in peak areas over time were monitored in three different solvents: water, 0,1 % formic acid, and 0,1 % acetic acid. The analytes were stable in both acids. Finally, 0,1 % formic acid was selected as the most suitable solvent for standards in the analysis on reversed phases and hydrophilic interaction liquid chromatography, as it was also used for acidifying the mobile phase.

Keywords: neurotransmitters, UHPLC-MS/MS, RP, HILIC, stability, DA, 5-HT, HVA, 5-HIAA, DOPAC, 3-MT, GABA, GLUT, AEA, 2-AG