

ABSTRACT

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Title of Doctoral Thesis: Development of method for the identification of alkaloids in plant extracts from Amaryllidaceae using UHPLC-MS/MS.

The purpose of this thesis is the development of method for identification of alkaloids from the family Amaryllidaceae in plant extracts using coupling of two methods UHPLC and MS/MS. The combination of methods UHPLC and MS/MS enables to work with the very small particles ($< 2 \mu\text{m}$) thereby fast separation with high efficiency and reduction of analysis time is achieved. MS is used for determination of atomic weight, molecular weight after the ionization of neutral species.

The development of methods was first conducted on UHPLC with PDA detection which served for the choice of optimal stationary and mobile phase. The tested columns were following: BEH PHENYL, BEH C18, BEH SHIELD RP18, HSS T3. The possibilities of separation and the behavior of the monitored substances at different pH (3 and 9), retention time and flow rate were studied. The column BEH SHIELD RP18 provided the best results of these columns, using buffer pH 3 and methanol as an organic modifier, and then flow rate 0.4 ml/min. Afterwards the new stationary phase CSH (charged surface hybrid) based on mixed-mode sorbents was introduced which is the interesting alternative for basic substances so it was included in the study.

The sample of extract of plant Zephyranthes and the standards of alkaloids (galanthamine, galanthine, haemanthamine, lycoramine, tazettine) were available. They were searched in the sample based on the knowledge of their molecular weight. The MS spectra of the standards and the sample and the fragmentation of the individual substances depending on the amount of the collision energy (15, 20, 30 V) were measured. The spectra of the sample were compared with the spectra of the standard substances. The presence of the searched alkaloids was confirmed. The next task was to find the presence other alkaloids except for known standards and to try to determine their identity.

Keywords: UHPLC – MS/MS, galanthamine, galanthine, haemanthamine, lycoramine, tazettine, BEH stationary phases, CSH stationary phases.