Abstract

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Title of Doctoral Thesis: Development of UHPLC-MS/MS method for separation of Amaryllidaceae alkaloids

In this master thesis, a method for the separation of 30 alkaloids of the Amaryllidaceae family was developed using ultra-high performance liquid chromatography coupled with tandem mass detection (UHPLC-MS/MS). Chromatographic conditions and mass spectrometer parameters were optimized.

In the first step of optimization, screening of 10 analytical columns was performed on an ultra-high performance liquid chromatography coupled with photodiode array detection (UHPLC-PDA). Those tested columns were Acquity UPLC BEH C18, Acquity UPLC CSH C18, Acquity UPLC BEH Phenyl, Acquity UPLC CSH Phenyl-hexyl, Arion Plus C18, Acquity UPLC BEH Shield RP18, Ascentis Express RP-Amide, ACE Excel C18-PFP, Kinetex PFP, and Kinetex F5. Acquity UPLC BEH Phenyl, Acquity UPLC CSH Phenyl-hexyl, and Acquity UPLC CSH C18 as they provided the highest separation score for the analyzed isomeric groups of analytes were selected for further optimization.

The optimization of MS conditions was performed by adjusting the parameters of the ion source and setting SRM transitions. The separation was optimized by testing the pH of the mobile phase, gradient slope, analysis time and column temperature utilizing UHPLC-MS/MS. In further step the effect of an organic modifier was also tested, where a mixture of MeOH:ACN (1:1, 1:2, 2:1, 1:3, 3:1) was used instead of pure MeOH.

The developed UHPLC-MS/MS was validated in terms of precision, selectivity, quantitation limit and linearity. Furthermore, analysis of the extract from dried leaves of *Crinum asiaticum*, Amaryllidaceae family, and the extract from dried bulbs of the genus *Narcissus*, Amaryllidaceae, was conducted.

Keywords: alkaloids of the Amaryllidaceae family, UHPLC-MS/MS, method development, optimization, validation, PDA detection