ABSTRACT (EN)

Proteinogenic amino acids are key components of living organisms. Thus, the latest metabolomics research has focused on developing fast and sensitive methods for the determination of amino acids. In this context, this thesis contains two studies describing development of high-performance separation techniques for the quantification of amino acids.

In the first study, a capillary electrophoresis method was developed for the determination of free amino acids in tobacco plants, particularly focusing on optimizing the extraction of amino acids from solid plant materials. The extraction procedure was optimized using design of experiments (DoE) to obtain the highest possible extraction yield of amino acids. Factors such as volume and concentration of the extraction solvent (hydrochloric acid) were assessed as the most significant. Subsequently, the optimal values of these factors were determined using response surface methodology (RSM). Lastly, proteinogenic amino acids were quantified using capillary electrophoresis with contactless conductivity detection and calibration with internal standard, which improved the precision of the method.

The second study aimed at developing a supercritical fluid chromatography method for the determination of free proteinogenic amino acids in human plasma. The most important part of this study was to improve the solubility of proteinogenic amino acids in a CO_2-rich mobile phase. Firstly, the polarity of mobile phase was increased by adding water and ammonium formate to the CO_2/methanol mixture. Secondly, the polarity of amino acids was decreased by derivatization with 1-chlorobutane. The derivatization step greatly enhanced the solubility of amino acids in the mobile phase, thus substantially improving the shapes of the amino acid peaks. Proteinogenic amino acids were quantified using tandem mass spectrometry detection and a calibration curve with deuterated internal standards, which provided an acceptable method precision.

In this thesis, both methods were compared, focusing on separation efficiency, limits of quantification, and acquisition and operating costs. This comparison showed that the separation efficiency was better in capillary electrophoresis method than in supercritical fluid chromatography. On one hand, the latter was faster than the former and enabled the quantification of concentrations up to five orders of magnitude lower. On the other hand, operation of a supercritical fluid chromatography with tandem mass spectrometry not only

requires more qualified operators but also more stable laboratory conditions and higher acquisition and operating costs.