

7 Summary

The presented dissertation thesis deals with an extensive topic of natural compounds analysis by the means of modern separation techniques described from different points of view.

Firstly, theoretical information about studied natural compounds (mainly antioxidants) has been summarized including characterization of the mechanism of free radicals formation and development of oxidative stress in human organism. Furthermore, the function of antioxidants and key roles of antioxidant therapy have also been discussed. Within the analyzed antioxidants, biological and physicochemical properties of phenolic acids, vitamins A and E and polyunsaturated fatty acids are described. Concurrently, known and published analytical procedures applied on each group of compounds are summarized. Besides antioxidants, content substances of yellow gentian (dried roots of *Gentiana lutea*) have been analyzed. Information about gentian pharmacological action, its practical utilization in therapy and ways of analytical determination represents the last chapter of theoretical part dealing with the studied compounds. The second point of view comprises characterization of individual separation techniques used in the thesis. Basic principles of gas chromatography (GC), solid phase microextraction connected to GC (SPME-GC), Ultra performance liquid chromatography (UPLC), mass spectrometry (MS) and capillary electrophoresis (CE) are summarized in the second part of thesis theory. Third point of view includes experimental work and particular projects dealing with the analysis of studied compounds. Analysis of chloroformate derivatives of phenolic acids by the means of SPME-GC is firstly presented. Developed method was published in *Analytica Chimica Acta* magazine, the full text is listed in Chapter 5.3. Furthermore, gas chromatography was utilized for determination of fatty acids in nutraceuticals containing encapsulated fish oil. Results were presented in a form of a poster that is stated in Chapter 5.10. UPLC, a modern representative of liquid chromatography, was used for development and validation of a novel method for simultaneous determination of vitamin A (retinol) and vitamin E (α -tocopherol) in human serum. The developed assay was compared with an HPLC method using particulate and monolithic columns within a publication in *Analytical and Bioanalytical Chemistry* magazine (See Chapter 5.4). Another experiment comprised UPLC-MS separation of more

172 complex mixture of vitamins A and E containing retinol, retinoic acid, α -, γ - and δ -tocopherol. The achieved results were presented as two posters that are also stated in Chapters 5.11 and 5.13.

The last topic comprised determination of gentisin, isogentisin and amarogentin in yellow gentian by capillary electrophoresis. This project was studied during a study stage at the University of Innsbruck. As a result a short communication was published in the *Journal of Separation Science*. The full text is listed in Chapter 5.6.