ABSTRACT

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Title of Doctoral Thesis: Application of Ultra-High Pressure Liquid Chromatography with Mass Spectrometry Detection in Analysis of Polyphenols

The major focus of presented doctoral thesis is development of advanced separation methods utilizing liquid chromatography (LC) and mass spectrometry (MS) for the analysis of polyphenols. The general aspects of techniques are discussed in theoretical part and the actual practical impact is documented by the results published in four original articles appended in the supplement I-IV. LC was used in each published study, while combined with MS in the last two works (supplement III-IV). Thus, the semi-quantitative data acquired by emerging laser desorption/ionization (LDI) MS technique were confirmed by UHPLC-MS. The broad availability, versatile configuration, convenient handling and well understood principles make LC the “method of choice” for variety of scientific applications to analyze diverse analytes. LC-MS coupling is convenient and robust offering two-staged separation: i) chromatographic separation and pre-concentration into narrow bands using LC and ii) separation based on mass-to-charge ratio (*m/z)* performed in the MS instrument. Additional selectivity and/or structural information, which can be obtained through MS detection, exceed any other on-line LC detection technique. The advantages of UHPLC over conventional HPLC for the analysis of polyphenols (PPs) in tea and wine were evaluated (supplement I), while UHPLC methods typically offered higher separation efficiency, together with substantial reductions in run time and solvent consumption compared to HPLC. The red wine samples were also analyzed using LDI-MS technique with previous solid phase extraction (SPE) sample work-up (supplement IV). LDI-MS application

shows an example of highly accurate molecular weight and mass resolution measurements sufficient for identification of analytes in complex mixture purely based on MS data, without prior LC separation. However, the sensitivity, selectivity and versatility of assay are greatly improved with prior LC separation step also giving more reproducible and quantitative results. Thus, UHPLC system was coupled with tandem MS detection for the analysis of catehins contained in green tea (supplement III).

In general, studies overlaying this doctoral thesis demonstrates that careful attention to the fundamentals of physical chemistry provide solid insights that can greatly facilitate the development of novel analytical methods and emerging applications in the analysis of polyphenols.