Combined Analytical Techniques For the Analysis of Complex Consumer Products and Bio-Samples G. G.Chen

Summary

Analytical chemistry plays a critical role in many fields ranging from fundamental research to life sciences, industrial analysis and social applications. The aim of this thesis is to develop new analytical methods to meet the ever-increasing need for safety and quality control, performance evaluation, claim substantiation and mode-of-action understanding in the area of consumer products for foods, and home and personal care. In **Chapter 1**, the needs and challenges of analytical sciences in the industry of foods and HPC are summarized. The most widely used analytical techniques in these product fields are introduced. Finally, the scope of this thesis is discussed with a brief introduction of the subsequent chapters.

In **Chapter 2**, a multi-residue method was developed for rapid determination of pesticide residues in tea by UHPLC-MS/MS. An adapted QuEChERS method was used for sample preparation. In order to minimize the matrix effects from tea, an SPE cartridge layered with graphite carbon/aminopropylsilanized silica gel was applied as complementary to the original QuEChERS method. Representative matrix-matched calibration curves were applied for quantification to compensate for matrix effects. Limits of quantification varied for the different pesticides. Except for dichlorvos, that has a quantification limit of 0.02 mg/kg, all others can be measured at 0.01 mg/kg level or better in a 5 g tea sample. Recoveries ranged from 70% to 120% and the method RSD met the European Union Quality Control guideline. Efficiency and reliability of this method were investigated by the analysis of both fermented and unfermented Chinese tea samples. The method has further application opportunities, including the analysis of e.g. dried vegetables and herb extracts.

In **Chapter 3**, a UHPLC-UV method combined with SPE sample pre-treatment was developed and validated for the rapid quantification of L-theanine in ready-to-drink (RTD) teas. UHPLC-UV analysis of twenty-seven RTD teas from the Chinese market revealed that the L-theanine levels in various types of RTD teas were significantly different. RTD green teas were found to contain the highest mean L-theanine level (37.85 ± 20.54 mg/L), followed

by jasmine teas (36.60 ± 12.08 mg/L), Tieguanying teas (18.54 ± 3.46 mg/L) black teas (16.89 ± 6.56), Pu-erh teas (11.31 ± 0.90 mg/L) and Oolong teas (3.85 ± 2.27 mg/L). The ratio of the total polyphenols to L-theanine content could be used as a characteristic parameter for differentiating RTD teas. L-theanine in RTD teas could be a reliable quality parameter that is complementary to total polyphenols.

In **Chapter 4,** the noncovalent interaction between β-CD and EGCG was studied at the molecular level by ESI-MS and NMR. Inclusion complexation of β-CD and green tea catechins was observed by ESI-MS. The stoichiometry of the β-CD-EGCG complex was determined using Job's method, which showed a maximum at 0.5, indicating a 1:1 stoichiometry of the β-CD-EGCG complex. NMR experiments indicated that inclusion complexes of β-CD and EGCG were formed and that the D ring or B ring of the EGCG molecule penetrated into the β-CD cavity. This molecular encapsulation could prevent the gallate moiety from binding to the human taste receptors, in that way reducing the bitter, astringent taste of EGCG. The direct observation of non-covalent interactions makes the combined deployment of ESI-MS and NMR a valuable chemical vehicle for fast screening of molecular maskers for reducing bitterness and astringency of green tea catechins as an alternative to a tasting panel.

In **Chapter 5,** a sensitive and specific UHPLC-MS/MS method was developed and validated for the measurement of climbazole (CBZ) deposition from hair care products onto artificial skin and human scalp. Deuterated CBZ was used as the internal standard. APCI in positive mode was applied for the detection of CBZ. For quantification, MRM transition 293.0 > 69.0 was monitored for CBZ, and MRM transition 296.0 > 225.1 for the deuterated CBZ. The linear range ran from 4 to 2000 ng/mL. The LOD and the LOQ were 1 ng/mL and 4 ng/mL, respectively, which enabled quantification of CBZ on artificial skin and human scalp at ppb level (corresponding to 16 ng/cm^2). For the sampling of CBZ from human scalp the buffer scrub method using a surfactant-modified PBS solution was selected based on a performance comparison of tape stripping, the buffer scrub method and solvent extraction in *in vitro* studies. Using this method, CBZ deposition in *in vitro* and *in vivo* studies was successfully quantified.

In **Chapter 6**, a sensitive UHPLC-MS/MS method has been developed and validated for simultaneous quantification of Zinc pyrithione (ZPT) and CBZ deposited onto human scalp from AD shampoos. Scrubbing with a buffer solution was used as the sampling method for the extraction of ZPT and CBZ from scalp. Derivatization of ZPT was carried out prior to UHPLC-MS/MS analysis. The identification of ZPT and CBZ was performed by examining ratios of selected MRM transitions in combination with UHPLC retention times. The limit of detection for ZPT and CBZ was established to be 1 and 2 ng/mL, respectively. This sensitivity enables the quantification of ZPT and CBZ at deposition levels in the low ng/cm^2 range. The method was successfully applied for the analysis of scalp buffer scrub samples from an *in vivo* study. The levels of ZPT and CBZ remaining on the scalp at different time intervals after application of the AD shampoo were measured. The results revealed that dual-active AD shampoo delivered more ZPT onto the scalp in a single wash than a single active shampoo did. The amount of ZPT and CBZ remained on the scalp after AD shampoo application declined over 72 hours. The method is also applicable in other studies, e.g. in artificial skin studies to improve shampoo formulations to maximize ZPT and CBZ deposition.

In **Chapter 7**, a method involving scalp cyanoacrylate biopsy sampling, a tailor-made cutting device, UHPLC-MS/MS analysis, SEM measurement and Raman imaging are described for the measurement of delivery of ZPT and CBZ from an AD shampoo into the scalp follicular infundibulum. Scalp cyanoacrylate biopsy enables the sampling of ZPT and CBZ delivered into the scalp follicular infundibulum as well as onto the scalp surface. Raman imaging of scalp cyanoacrylate biopsy samples allows the visualization of the spatial distribution of ZPT and CBZ deposited on the scalp. A tailor-made cutting device enables the separation of the scalp follicular infundibulum sample (20 μm below the scalp surface) from the scalp surface samples (including the top 20 μm of the infundibula). UHPLC-MS/MS was used as a sensitive and specific methodology enabling the quantification of ZPT and CBZ without interferences. Using this method, ZPT and CBZ delivered into the scalp follicular infundibulum from the dual-active AD shampoo was successfully visualized and quantified.

Due to the lipophilic nature of CBZ and hence the increased solubility in sebum, CBZ has the ability to penetrate further into the sebum-rich infundibulum whereas ZPT remains within the upper 20 µm of infundibula. This differential distribution of actives allows for the effective targeting of *Malassezia* species throughout the depth of the scalp follicular infundibulum.

Finally, **Chapter 8** proposes an *ex vivo* method that combines tape strip sampling and scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDX) for measuring and visualizing the particle size, morphology and composition of ZPT deposited onto the scalp from an AD shampoo containing ZPT and zinc carbonate. Hair was washed with a commercially available AD shampoo containing ZPT and zinc carbonate ($ZnCO_3$). Tape strips were applied to collect the deposited particles from the scalp after AD shampoo application and rinse-off. The scalp tape strip samples were subjected to scanning SEM/EDX measurement. The morphology of the ZPT particles was visualized by SEM imaging and identification of ZPT particles was confirmed by EDX analysis. For the commercial shampoo studied it was observed that two types of zinc-containing particles with different morphologies and composition remained on the scalp after shampoo application and rinse-off. As indicated by the EDX spectra, the ZPT particles deposited onto the scalp surface had polygonal crystal structures. $ZnCO_3$ was also deposited onto the scalp surface. This material was mainly present as aggregated particles. This *ex vivo* measurement method provides higher imaging resolution and more chemical specificity than reflectance confocal microscopy. To the best of our knowledge, this is the first time that ZPT particles could be distinguished from other zinc-containing particles deposited onto the scalp. The new method allows the microstructures of both ZPT and other zinc particles on the scalp to be imaged.