DOCTORAL THESIS

Method development and applications of capillary electrophoresis, liquid chromatography and mass spectrometry for the separation and determination of urinary prophyrins

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Method Development and Applications of Capillary Electrophoresis, Liquid Chromatography and Mass Spectrometry for the Separation and Determination of Urinary Porphyrins

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Abstract

This thesis describes method development and applications of capillary electrophoresis (CE), high performance liquid chromatography (HPLC) and mass spectrometry (MS) for the separation and detection of six urinary porphyrins with biological significances, namely uroporphyrin (UP), heptacarboxylic acid porphyrin (HEPTAP), hexacarboxylic acid porphyrin (HEXAP), pentacarboxylic acid porphyrin (PENTAP), coproporphyrin (CP) and mesoporphyrin (MP). Excessive accumulation and excretion of porphyrins often result from metabolic aberration. The measurement of total contents and individual concentrations of the urinary porphyrins in biological materials, therefore, is essential for the confirmation and diagnosis of various diseases associated with porphyrins such as porphyrias and liver diseases.

In Chapter 2, stacking and separation of the six urinary porphyrins were demonstrated in micellar and microemulsion electrokinetic chromatography (MEKC and MEEKC). Adequate resolution and efficiency and enhanced sensitivity were obtained by the introduction of the sample containing acetonitrile (ACN) and high salt content (*ca*. 1% NaCl) into the CE capillary at more than 10% capillary volume. The enrichment factors ranged from 12–32 in MEKC and 28–33 in MEEKC. Calibration curves obtained for the determination of CP isomers were linear between 30–400 nM with $R^2 = 0.999$ and the limit of detection (LOD) was 20 nM in MEEKC. Intra- and inter-day precisions from the analyses of spiked urine samples at concentrations of 40–400 nM were 0.1–0.4% for migration time and 0.7–7.6% for peak area. CP-III,

CP-I and UP were detected at levels of 80.7, 32.3 and 19.8 nM, respectively, in the healthy human urine samples. Different porphyrin profiles were observed in urine samples from porphyria cutanea tarda (PCT) patients.

In Chapter 3, a lab-made liquid junction interface of CE-ESI-MS was developed for the analysis of MP, CP and UP. The intra-day precisions of the porphyrins for migration time and peak area were 1.1–3.9% and 2.3–14.1%, respectively. The correlation coefficients of calibration curves were 0.9956–0.9862. The recoveries from urine were 73–96% and the LODs determined for MP, CP and UP were 57, 125 and 260 nM, respectively. The interface was demonstrated to be applicable for the potential quantitative analysis of clinical samples.

In Chapter 4, an HPLC-ESI-MS method was presented for the separation and determination of the six urinary porphyrins. The intra- and inter-day precisions for peak area were 3.96–9.74% and 5.49–15.3%, respectively. The recoveries of the porphyrins from urine were between 83.6–108%. The LODs were 0.18–3.06 nM. Endogenous UP, HEPTAP and PENTAP were detected at 42.4, 21.3 and 18.0 nM, respectively. CP was predominantly present at 135.6 nM, while MP and HEXAP were not detected in the healthy female urine. Significant increases of CP excretion levels in urine from liver disease patients indicated that monitoring of CP in human urine might be served as a diagnostic symptom of liver diseases.

In Chapter 5, summary and comparison of the methods for the determination of urinary porphyrins were made in terms of the separation efficiency, LOD, accuracy and precision.

Table of Contents