Development of a solid-phase extraction method with simple MEKC-UV analysis for simultaneous detection of indole metabolites in human urine after administration of indole dietary supplement

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This work presents the development of a solid phase extraction method with simple MEKC-UV analysis for the simultaneous determination of indole-3-carbinol (I3C) and its metabolites (3, 3′-diindolylmethane (DIM), indole-3-carboxaldehyde (I3CAL), indole-3-acetonitrile (I3A)) in human urine after oral administration of an indole dietary supplement. Solid phase extraction (SPE) method was applied for the first time for simultaneous analysis of these indole metabolites. The MEKC separation method was developed in a previous work. Three commercial SPE cartridges, each with different sorbent materials, were investigated: Sep-Pak® C18, Oasis® HLB and Oasis® WCX. The Sep-Pak® C18 material provided the highest extraction recovery of 88–113% (n = 9), for the four target indole metabolites (I3C, DIM, I3CAL and I3A). The optimal washing and elution solutions were 40% methanol/water (v/v) and 100% methanol, respectively, and optimal elution volume was 2.0 mL. The specificity of the proposed SPE method was evaluated with negative control urine samples (n = 10) from healthy volunteers who had not taken the dietary supplement or vegetables known to contain indole compounds. Linear calibration curves were in the range of 0.2–25 μg mL⁻¹ (r² > 0.998) using diphenylamine (DPA) as the internal standard. Intra-day and inter-day precisions were 3.5–12.3%RSD and 2.7–14.1%RSD, respectively. Limits of detection and quantification were 0.05–0.10 μg mL⁻¹ and 0.10–0.50 μg mL⁻¹, respectively. The four target indole compounds were separated within only 5 min by MEKC-
UV analysis. Urine from 5 subjects who had taken a dietary supplement containing I3C and DIM were found to contain only the DIM metabolite at concentrations ranging from 0.10 to 0.35 µg mL$^{-1}$. Accuracy of the proposed method based on the percentage recovery of spiked urine samples were 70–108%, 82–116%, 82–132% and 80–100% for I3C, I3CAL, I3A and DIM, respectively. The Sep-Pak® C18 cartridge was highly effective in extraction and sample cleanup for the downstream simultaneous detection of urinary indole metabolites by MEKC-UV method.

Reference: