

A Facile Method for the Quantification of Urinary Uracil Concentration by a Uracil-Specific Fluorescence Derivatization Reaction.

Abstract

A facile and reliable fluorescence method for the quantification of urinary uracil concentration is proposed herein. The assay utilizes a specific fluorescence (FL) derivatization reaction for uracil using 3-methylbenzamidoxime as a fluorogenic reagent. Although the presence of urine inhibited the FL reaction, 10 μL of urine was sufficient for the detection of urinary uracil. The uracil derivative was successfully separated from other fluorescent impurities using simple reversed-phase LC with FL detection. Urinary uracil concentrations from 16 people were compared with the concentrations obtained by the traditional column-switching liquid chromatographic analysis with UV detection. The FL derivative of uracil appeared as a single peak in the chromatograms of all samples. However, several samples showed an additional peak overlapping the uracil peak when using the column-switching method because of UV-active impurities. These results indicated that the present method is not affected by interfering substances in urine and affords a precise determination of urinary uracil. We expect the proposed method to be applicable for diagnosing dihydropyrimidine dehydrogenase deficiency in 5-fluorouracil chemotherapy.