Ultra-high performance liquid chromatographic determination of aflatoxin M1 in urine

ABSTRACT

The development of analytical methods to detect aflatoxin B1 (AFB1) in foodstuffs and its metabolites in human biological samples is useful for risk assessment. The latter methodology, i.e. the measurement of AFB1 biomarkers, has become important to assess human aflatoxin exposure. AFB1-lysine adduct, AFB1-DNA adduct and urinary aflatoxin M1 (AFM1) are some of the AFB1 biomarkers that can be measured by several analytical methods, such as enzyme-linked immunosorbent assay, radioimmunoassay, and high performance liquid chromatography (HPLC). HPLC coupled to a fluorescence detector is useful and preferable due to its high degree of sensitivity, but the analysis may take time and consume large amount of solvents. Therefore, the present study extrapolated the HPLC method to ultra-HPLC for the determination of urinary AFM1. After the extraction procedure with an immunoaffinity column, chromatographic separation was done using a high performance 1.8 μm microparticulate C18 column. The mean recovery from urine samples spiked with 0.5, 1.0 and 2.0 ng/ml AFM1 was 84.4±4.0%, with acceptable recovery values, interday (6.0±5.3%) and intraday (2.6±0.6%) coefficients of variation. The retention time was 5.7 min. This method was used to measure urinary AFM1 in 71 subjects, of which 13 had AFM1 levels above the limit of detection (0.018 ng/ml). The mean urinary AFM1 level of the positive samples was 18.8±28.6 pg/ml, ranging from 2.4 to 100.4 pg/ml. As this is one of the few studies investigating the occurrence of aflatoxin biomarkers in human biological samples in Malaysia, a study with a larger sample size is necessary to investigate the magnitude of aflatoxin exposure among the population.

Keyword: Aflatoxins; Urinary aflatoxin M1; Ultra-high performance liquid chromatography; Malaysia