Abstract

Milk may contain aflatoxin M_1 (AFM_1) if cows ingest aflatoxin B_1（AFB_1）, which is a usual contaminant in animal feed. AFM_1 is toxic and carcinogenic to liver, and can survive after pasteurization. The European Union has reduced the regulated level down to 0.05 μg/L and has challenged the existing analytical methods.

To confirm the existence of aflatoxin M_1, the most used measurement is by high-performance liquid chromatography (HPLC)/fluorescence detection. Usually derivatization is needed to enhance the fluorescence of aflatoxin M_1, which needs toxic reagent and additional time. In terms of sample preparation, the sample size is limited to 20–50 mL because a traditional solid-phase adsorbent only allows slow flow, which confines the sensitivity.

This study improved the sensitivity by using a disk type of solid-phase adsorbent and detection by high-performance liquid chromatography/tandem mass spectrometry. We successfully increased the sample volume up to 200 mL without significant breakthrough. The recoveries and detection limits are as follows: (1) recoveries using immunoaffinity-column (IAC) cleanup in milk powder, whole milk, and low-fat milk were 67.9 ± 9.3%、78.2 ± 7.3% and 86.6 ± 4.1% (mean ± SD), respectively; the method detection limits（MDL）in whole milk and low-fat milk were 0.59 ± 0.19 ng/L（1.02 ng/L）and 0.66 ± 0.12 ng/L（0.94 ng/L）（mean ± SD（MDL at 99% confidence level, $MDL_{99\%}$））, individually. (2) Recoveries using multifunctional cleanup columns (MFC) in whole milk and low-fat milk were 6.51 ± 0.28% and 15.8 ± 4.6% (mean ± SD), respectively; their method detection limits were 14.1 ± 2.6 ng/L （24.6 ng/L）and 9.22 ± 3.34 ng/L（16.8 ng/L）（mean ± SD（$MDL_{99\%}$））, individually. The

method detection limits of certified reference material BCR 282 milk powder for IAC and MFC were 0.0085 ± 0.00050 μg/kg （0.0096 μg/kg） and 0.0082 ± 0.0022 μg/kg （0.013 μg/kg）（mean ± SD（MDL 99%））, respectively. Recoveries between the two clean-up methods were significantiy different.

This research tried to synthesize stable isotope-labelled aflatoxin M_1 as the internal standard. We successfully exchanged ^{16}O on the AFM_1 for ^{18}O; however, the reaction got equilibrium and the nonreacted ^{16}O-AFM_1 will cause false positive. Furthermore, the syntheses of deuterium-labelled AFM_1 were a basic solution and high reaction temperature, which resulted in decomposition of the compound. Consequently, these two approaches were both failed. On the other hand, we tested the suitability of AFB_1 as a recovery standard, but the linearity of the calibration curve was worse than the one built up based on external standard. Thus, AFB_1 is not a good recovery standard in our investigation.

Our procedure improved the detection limit that is 3- to 500- fold lower than existing methods, which provides an extremely sensitivity to milk analysis of AFM_1.

Keywords: solid-phase extraction, immunoaffinity column, multifunctional cleanup column, certified reference material, high-performance liquid chromatography / tandem mass spectrometry