Abstract

Feedstuff are subject to the pollution of *Aspergillus*, which is able to produce aflatoxin B1 (AFB1). After injestion by lacting cow, AFB1 can be transfered into aflatoxin M1 (AFM1) and is secreted in milk, which is carcinogenic and toxic to liver. AFM1 has been classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) since 2002. Unfortunately, AFM1 is relatively stable either in preparation and storage of milk products.

The European Union has reduced the AFM1 maximum allowance from 0. 5 μg/L to 0.05 μg/L since 1999, and this has become a challenge for most of the existing analytical methods. The official analytical method of Bureau of Food and Drug Analysis, Taiwan, processes milk samples with immunoaffinity column (IAC) and detects AFM1 using high-performance liquid chromatography coupled with a fluorescence detecter (HPLC/FLD). The aims of this study were to modify pre-treatment method in order to shorten analytical time and improve detection limits. There were two strategies in our study. The first one was to simplify the milk sample preparation using restricted access materials (RAM) pre-column and shodex column. The second one was to increase the throughput and sensitivity with ultra performance liquid chromatography (UPLC). Three UPLC columns were compared with a traditional HPLC C_{18} column.

RAM pre-column did not retain the AFM1 in milk, and the proteins in milk were co-eluted with AFM1. Thus, RAM pre-column was not suitable for the sample preparation. Shordex column can separate the proteins in milk with AFM1 but the peak width of AFM1 is too broad (>3 min). Consequently, we still used IAC columns for sample preparation.

AFM1 was analyzed using an Acquity UPLC system and tandem mass

spectrometry with positive electrospray ionization. The HSS T3 UPLC column gave the best performance on selectivity and sensitivity. The on-column detection limit of AFM1 was 0.011 ng/mL (0.11 pg) of AFM1 and the method detection limit of AFM1 in milk powder was 2.01 ng/kg. The ion suppression was 63.4% for milk； therefore, matrix-matched standard curves were set up to eliminate the matrix effect. A good linearity was obtained with the square of correlation coefficient larger than 0.997. Aflatoxin G1 is not a good internal standard , which influenced the quantification of AFM1.

Use of UPLC lowed the detection limit (0.11 pg), shortened the run time (2.6 min), and the signal-to-noise ratios increased 16−58 times than the HPLC column because of their narrow peaks.

Key words：aflatoxin M1 (AFM1), aflatoxin G1 (AFG1), immunoaffinity column (IAC), ultra performance liquid chromatography tandem mass (UPLC MS/MS), restricted access materials column (RAM pre-column), Shodex column, milk