Development and validation of a sensitive monoclonal antibody-based ic-ELISA for the aflatoxin M_1 in milk (Abstract)

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ABSTRACT

Aflatoxin M_1 (AFM_1), a hydroxylated metabolite of aflatoxin B_1 (AFB_1), possesses the carcinogenic, mutagenic, and toxic activities. To protect the health of animals and humans, it is clear that the need for an effective residue-monitoring programme to detect the AFM_1 residues. In this study, a modified procedure was used to prepare the hapten AFB_1 and AFM_1 derivatives. Then, the prepared antigen AFM_1-CMO-KLH was used to inoculate female Balb/c mice to prepare a sensitive monoclonal antibody (mAb) against AFM_1. After cell fusion and culture several times, the hybridoma cell line, 3D8, which was of the IgG1 isotype, was selected to obtain a highly sensitive mAb. The obtained 3D8 mAb displayed an IC_{50} value of 64.75 ng L^{-1} for AFM_1 and did not exhibit measurable cross-reactivity with other aflatoxins and antibiotics. Based on this mAb, an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) was established that utilizes simple sample preparation and clean-up methods. The decision limit (CCα, α = 1%), detection capability (CCβ, β = 5%), and LOQ value for the AFM_1 matrix calibration method were 24 ng L^{-1}, 27.5 ng L^{-1}, and 35 ng L^{-1} in the milk matrices, respectively. The AFM_1 recovery ranged from 85.3% to 107.6%. The CVs were less than 13.8%. A positive correlation ($r > 0.99$) was observed between the ic-ELISA and HPLC-MS/MS results. This ic-ELISA would be a useful tool for screening the AFM_1 residues in milk.

Keywords: Monoclonal antibody (mAb), Aflatoxin M_1, milk.