

## Research Article

# Determination of Aflatoxin B<sub>1</sub> in Feedstuffs without Clean-Up Step by High-Performance Liquid Chromatography

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A reliable and rapid method has been developed for the determination of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in four kinds of feedstuffs comprising broken rice, peanuts, corn, and fishmeal. A sample preparation was carried out based on the QuEChERS method with the exclusion of the clean-up step. In this study, AFB<sub>1</sub> was extracted using acetonitrile/methanol (40/60 v/v), followed by partitioning with sodium chloride and magnesium sulfate. High-performance liquid chromatography with precolumn derivatization and fluorescence detection was performed. The coefficients of determination were greater than 0.9800. Throughout the developed method, the recovery of all feedstuffs achieved a range of 82.50–109.85% with relative standard deviation lower than 11% for all analytes at a concentration of 20–100 ng/g. The limit of detection (LOD) ranged from 0.2 to 1.2 ng/g and limit of quantitation (LOQ) ranged from 0.3 to 1.5 ng/g. The validated method was successfully applied to a total of 120 samples. The occurrence of AFB<sub>1</sub> contamination was found at the following concentrations: in broken rice (0.44–2.33 ng/g), peanut (3.97–106.26 ng/g), corn (0.88–50.29 ng/g), and fishmeal (1.06–10.35 ng/g). These results indicate that the proposed method may be useful for regularly monitoring AFB<sub>1</sub> contamination in feedstuffs.

## 1. Introduction

Aflatoxins (AFs) are secondary metabolites of fungi (e.g., *Aspergillus flavus* and *A. parasiticus*). AFs occur naturally and can be found in common food and feedstuffs such as rice, peanuts, corn, fishmeal, and soybean meal [1–3]. The four major analogues—*aflatoxins B*<sub>1</sub> (AFB<sub>1</sub>), B<sub>2</sub> (AFB<sub>2</sub>), G<sub>1</sub> (AFG<sub>1</sub>), and G<sub>2</sub> (AFG<sub>2</sub>)—are the most important members because they all pose a potential risk to human and animal health if food and feedstuffs have been contaminated. In particular, the toxicity of AFB<sub>1</sub> can range from levels that may cause immune system suppression to the induction of teratogenic, mutagenic, and carcinogenic activities [4, 5], which is collectively classified as a carcinogen (Group 1) by

the International Agency for Research on Cancer (IARC) [6]. In addition, feedstuffs comprise the first link of the food chain; therefore, there is a risk of AFB<sub>1</sub> carryover from feedstuffs into animal tissues and/or biological fluids such as meat, milk, and eggs, which may eventually be hazardous for human consumption [7–9]. As a result of such adverse health effects of the toxin, it is necessary to have a sensitive, reliable, and accurate method for monitoring AFB<sub>1</sub> level in feedstuffs.

High-performance liquid chromatography with fluorescence detection (HPLC-FLD) is considered as the most reliable instrument for the quantification of AFs due to its accuracy and high sensitivity. However, it requires clean-up steps involving immune affinity columns (IAC) to remove interferences and preconcentration of AFB<sub>1</sub>. IAC is also