

Process Validation and Determination of Aflatoxins in Ground Chilli (*Capsicum annum L.*) Using LC-MS/MS Method

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Aflatoxins (AFs) are naturally occurring mycotoxins, produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. As chilli is highly susceptible to contamination with AFs, it is important to identify a suitable analytical technique to determine AFs content in chilli. Aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂) are the available AFs in chilli. A study was conducted to validate a liquid chromatography tandem mass spectroscopy (LCMS/MS) method for simultaneous determination and quantification of AFs in chilli. Chilli samples were spiked at two levels with 4 ppb and 10 ppb of AFB₁ / AFG₁ (1 ppb and 2.5 ppb of AFB₂/AFG₂, respectively). Spiked samples were extracted with 60% methanol and diluted with Phosphate Buffer Saline and cleaned-up with immunoaffinity column, eluted with 100% methanol. The quantification was carried out with Multiple Reaction Monitoring Mode (MRM) in LCMS/MS with positive polarity. The average recoveries of the samples spiked at two levels were within the range of 70 – 110%, the regulatory limit set in Commission Regulation EC No 401/2006. Satisfactory accuracy and precision were obtained in inter-day and intra-day analysis except for AFG₂. The inter-day RSD obtained for AFG₂ was 21.9%, which is above the regulatory limit of 20%. Appropriate linearity was obtained for the calibration curves ranging from 0.5 – 20.0 ppb of AFB₂ / AFG₂ (2.0-80.0 ppb of AFB₁ / AFG₁) with R² > 0.998. The limit of quantification (LOQ) was below 5 µg/kg for all AFs. Therefore, the method can be applied for the analysis of real chilli samples with high reliability. The method was applied for analysis of 14 packed chilli samples collected from supermarkets and 11 samples were contaminated with AFB₁ above the Sri Lankan Standards (SLS) regulatory limit of 5 µg/ kg, while 8 samples were contaminated with total AFs above the SLS regulatory limit of 10 µg/ kg.

Key words: Aflatoxin, Immunoaffinity column, LCMS/MS, Validation