Development of a novel method for the simultaneous quantification of cyanotoxins and aflatoxin M1 in milk by LC-MS/MS

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Cyanotoxins are secondary metabolites produced by cyanobacteria, a varied group of Gram-negative photosynthetic bacteria, also known as blue-green algae. Under specific conditions (i.e. water temperature, light intensity, and nutrient levels), cyanobacteria can form blooms. This natural phenomenon is characterised by an accumulation of algal biomass and the possible release of cyanotoxins into aquatic ecosystems. Human exposure to cyanotoxins through contaminated food or contaminated drinking water can be associated with symptoms such as fever, convulsion, gastroenteritis, headache and, in rare cases, death. Global warming has led to an increase in the duration and frequency of blooms and the use of contaminated water as drinking water for cattle could lead to the production of contaminated milk.

Aflatoxin M1 (AFM1) is a well-known mycotoxin, found as a metabolite of aflatoxin B1 (AFB1), present in the milk of animals after ingestion of feed contaminated with AFB1. Unlike cyanotoxins, for which there is no legislation, AFM1 levels in milk are regulated under Commission Regulation (EC) No 1881/2006.

To our knowledge, there is no existing LC-MS/MS method for the quantification of multiple cyanotoxins in milk. In this study, a simple and sensitive analytical method based on ultra-high-pressure liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) was developed, optimised and validated for the simultaneous quantification of AFM1, eight microcystins congeners, and nodularin. After improving the extraction and purification conditions (e.g. solvents, solid phase extraction), the parameters of the validation were assessed. Specificity and selectivity, limits of detection and quantification, linearity, matrix effect, recovery, reproducibility, repeatability and measurement uncertainty were determined in accordance with the main analytical guidelines.

Keywords: cyanotoxins, mycotoxins, LC-MS/MS, analysis, food control

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