Evaluation of measurement uncertainties of an analytical method for the determination of aflatoxin M₁ in milk and milk powder

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Abstract

The mycotoxin aflatoxin M₁ (AfM₁) is a serious food safety hazard for which the European Commission has already established a maximum permissible level of 0.05 μg/kg AfM₁ in milk and products thereof. For control analysis laboratories are increasingly asked to submit full uncertainties of their analytical results.

The evaluation of measurement uncertainties of an analytical method for the determination of AfM₁ in milk and milk powder on the basis of ‘in-house’ validation data in compliance with the ‘Guide to the Expression of Uncertainty in Measurement (GUM)’ [1] and the ‘EURACHEM Guide’ [2] is described. A similar approach will be used to assess the performance of methods employed by laboratories participating in the certification of reference materials for AfM₁ in milk powder.

Keywords: Aflatoxin M₁, milk, milk powder, uncertainty

Introduction

Aflatoxins are a group of mycotoxins, secondary fungal metabolites, which are of greatest significance in foods and feeds. The aflatoxin M₁ (AfM₁) is hydroxylated derivative of AfB₁, which is formed and excreted in the milk of lactating animals after the ingestion of AfB₁ contaminated food and feed. The frequent detection of AfM₁ in commercial milk and dairy products, the high consumption of these products, especially in infants populations and the probable carcinogenicity of AfM₁, led to an increasing public awareness and therefore to the establishment of measures to control the AfM₁ contamination of food- and feed-stuffs. The importance of AfM₁ as a food safety hazard is reflected in the existence of a maximum permissible level of 0.05 μg/kg AfM₁ in milk and milk products established by the European Commission [3] and additional 17 national regulations.
The analytical difficulty and the economic importance of controlling AfM1 levels in milk and dairy products led the BCR to prepare a series of certified reference materials (CRM) for AfM1 [4,5]. Because of the good acceptance of these CRMs it was required to prepare a new batch in 2001. In order to support the preparation and recertification of the CRMs it was necessary to establish an 'in-house' method for the determination of AfM1. In the following the calculation of the uncertainty of the method on the basis of validation data is described.

**Method**

The method for the determination of AfM1 in milk and milk powder is based on the ISO 14501 with minor modifications. The analyte is extracted by passing the test portion through an immunoaffinity column (IAC). The antibodies of the column bind selectively with AfM1 and form an antibody-antigen complex. Other components of the sample matrix are washed off. After the elution of the AfM1 and a concentration step the separation and quantification is carried out by reversed-phase high performance liquid chromatography (RP-HPLC) by fluorescence detection (FLD) respectively.

**Results and Discussion**

Uncertainty arises mainly from 5 sources, namely the concentration of the calibrant, calibration, sample reconstitution (weighing, redissolving and diluting), precision (varying operators, standards and times) and the recovery.

The uncertainty contribution of the AfM1 calibrant was determined by UV-spectrophotometry according to the ISO 14501 [6] and comparison with the results obtained for the determination of RM-423 (AfM1 in chloroform). Based on the spectrophotometric measurements an uncertainty (uc(std)) of 0.32 % was assigned to the concentration of the AfM1 calibrant.

The combined sample reconstitution uncertainty (uc(reconst)) of 0.1% comprises the uncertainties introduced by the weighing, redissolving and dilution of the milk powder.

The uncertainty of the weighing consists of the repeatability and accuracy of the balance. Redissolving and dilution was performed by adding defined volumes of water. The uncertainty introduced by both pipette (uc(pip)) and volumetric flask (uc(flask)) consists of the accuracy, repeatability and the volume expansion of the water.

The precision was checked by determining the repeatability and reproducibility for 2 concentration levels corresponding to 0.1 and 0.4 μg/kg AfM1 in milk powder respectively. The repeatability and corresponding uncertainty (u(repeat)) was calculated on the basis of 6 injections for both concentration levels on 3 different days. The reproducibility and the corresponding uncertainty (u(reprod)) was calculated by ANOVA for both concentration levels and based on the between days results on the 3 days of measurements. The uncertainties of the repeatability and reproducibility were combined to obtain the uncertainties of the precision (u(prec)). Combined uncertainties of the precision (uc(prec)) of 2.0 % and 1.2 % were calculated for the lower and higher concentration level respectively.

External standard calibrations of AfM1 were performed by 6-fold injections at 5 concentration levels (1.15-13.81 ng/mL AfM1) on 3 different days. The calibration