PREPARATION OF REFERENCE MATERIALS TO AFLATOXINS IN STANDARD SOLUTION AND PEANUT

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Reference materials are an important tool in realizing a number of aspects of measurement quality and are used for method validation, calibration, internal quality control and external quality assurance (proficiency testing) purposes. Also, it's highly recommended according to the ISO/IEC 17025 [1]. Different types of reference materials are required for different functions, in food analysis, more commonly are standard solutions and matrix reference materials. Certified reference materials distributed in proficiency testing need to be sufficiently homogeneous, and the requirement for sufficient homogeneity suggests the use of a formal test. Such tests as have been formulated rely on the duplicated analysis of material from a number of portions, followed by analysis of variance. But it's not simple, because if the analytical method used is very precise, then an undue proportion of material will be found to be significantly heterogeneous. If it is too imprecise, the test may be unable to detect heterogeneity [2]. In Brazil, there is few productors these materials, mainly to mycotoxin. The aim of this work was prepared peanut and standard solution reference materials for aflatoxins and the evaluation of the homogeneity and stability results by ISO GUIDE 35 [3]. In the preparation of raw peanut reference material, 3 Kg of sample naturally contamination with AFB_1 was pulverised in a hammer mill for 5 min and then it was transferred to homogenizer (CONSERLI MP20) for 15min. The method used for determination of aflatoxins in peanuts was High Performance Liquid Chromatography with post column derivatization [4]. For the homogeneity study, 10 samples (~ 6.0 % of the total batch, 155 bottles) of material were chosen using a random stratified sample picking scheme and analysed for their aflatoxins contained into material in duplicate. In the preparation of reference materials in solution, initially stock solutions were prepared from the crystalline toxins (B_1, B_2, G_1 and G_2) and then these were diluted in acetonitrile to nominal concentration around 10 μg/ml. After the check of the target concentrations, 1.5 mL of each calibrant was filled in amber glass ampoules. The standard solutions concentrations were determinate by UV spectrophotometry, using molar absorptivities. For homogeneity study, 5 ampoules of each toxin (~ 5 % of the total batch) were chosen using randon strafied sample picking and analysed in triplicate. The isochronous method was applied for stability study, where 3 samples of each mycotoxin solution were maintained in the temperatures of 4 and 37 ºC, along 10, 20 and 30 days and then transfered to reference temperature (-20 °C) until analysis. The homogeneity results were evaluated by a one-way analysis of variance (ANOVA). All materials were considered homogeneous, where $F_{calculated} < F_{critical}$, at a 95 % level of confidence, except of aflatoxina B_1 solution. In this case the heterogeneity can be explained by the high precision of analytical method and as aflatoxins in acetonitrile are considered a true solution, the heterogeneity of the material was negligible. No significant slope at 95 % level of confidence was detected for any material in the stability study, demonstrating that the materials were stable for the period studied and for this reason suitable to be used in proficiency tests.

References:

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