

**AOAC TDLM Method Verification Workshop
Determination of Aflatoxin B1 in Peanut Butter**



122nd AOAC
ANNUAL MEETING & EXPOSITION

**Aflatoxin B1 and Total Aflatoxins in Peanut Butter,
Pistachio Paste, Fig Paste, and Paprika Powder (AOAC
Official Method 999.07)**

**Immunoaffinity Column Liquid Chromatography with Post-Column
Derivatization**

Activity:

The lab is verifying the method for the determination of aflatoxin B1 in peanut butter. Use the ALACC Method Verification Guide and information from the AOAC collaborative study to plan how the lab should verify the method.

Verification Purpose

The lab has to provide evidence that it can achieve the performance characteristics stated in the standardized method (AOAC 999.07).

A. Principle

Test portion is either extracted with MeOH-H₂O (8 + 2) or MeOH-H₂O (8 + 2) plus hexane (or cyclohexane). Extract is filtered, diluted with water to a specified solvent concentration, and applied to an affinity column containing antibodies specific to aflatoxins B₁, B₂, G₁, and G₂. Aflatoxins are removed from the affinity column with MeOH and are quantified by reversed-phase liquid chromatography (LC) with post-column derivatization (PCD) involving bromination. PCD is achieved with either electrochemically generated bromine (Kobra cell) or with pyridinium hydrobromide perbromide (PBPB) and determined by fluorescence detection.

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D. Reagents

(p) *Aflatoxin standard solutions for LC.*—(1) *Mixed aflatoxins calibrant solution X for LC.*—Prepare as in 971.22B–E (see 49.2.03) to contain 1000 ng B₁, 200 ng B₂, 1000 ng G₁, and 200 ng G₂/mL toluene–acetonitrile (98 + 2). (2) *Working calibrant solutions for LC.*—Prepare solution by pipetting exactly 2.0 mL calibrant solution X into 20.0 mL volumetric flask (or 2.5 mL into 25 mL volumetric flask). Dilute to mark with toluene–acetonitrile solution and shake well. Use this solution for pipetting the volumes listed in Table 999.07B into a set of 10.0 mL volumetric flasks. Evaporate toluene–acetonitrile solution just to dryness under stream of N at room temperature. To each flask, add 4 mL methanol, mix, dilute to 10.0 mL with water, and mix again. Prepare working solutions daily.

Table 999.07B. Preparation of working calibration solutions

Working standard	Aliquot taken from working calibrant solution, μL	Final mass
		B ₁
1	40	0.400
2	120	1.200
3	200	2.000
4	280	2.800
5	360	3.600

AOAC Collaborative Study Information

Results are presented from a collaborative study of a method for the determination of aflatoxin B₁ and total aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder. Per matrix type fortified and naturally contaminated samples were tested by 12-16 laboratories participating in the trial.

Table 999.07A. Interlaboratory study results for aflatoxin B₁ and total aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder

Food	Contamin.	Avg., ng/g	No. labs ^a	Aflatoxin B ₁				
				s _r	RSD _r , %	s _R	RSD _R , %	HorRat
Peanut butter	Fortified	0.9	15	0.09	10	0.16	19	0.42
	Fortified	3.6	13	0.11	3	0.66	18	0.49
	Naturally	0.8	15	0.05	6	0.26	32	0.69
	Naturally	1.5	14	0.10	6	0.22	14	0.33
	Naturally	3.4	14	0.13	4	0.65	19	0.51