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Pons Scaled-Down Clean-Up Column Adapted for Use in Solvent-Saving Modification of the CB Method for Aflatoxin

L.S. LEE and **E.A. CATALANO**, Southern Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, New Orleans, LA 70179

ABSTRACT

The solvent-saving procedure devised by Pons using a small chromatographic tube (Bio-Rad Laboratories glass Econo-Column, 10 mm id × 300 mm long) has been adapted and extended for use in modifications of the Official AOAC procedure for quantitative determination of aflatoxins in corn, peanuts, soybeans, coconut and pistachios. Thirty mL of each of 3 solvents for column washes was used instead of the 150 mL specified by the Official CB Method. The analytical aliquot was also reduced 80%, but sample size and extracting solvent volume were not changed, so that there was no loss in sensitivity. Toxins ranging from 3 to over 1,000 μg/g of sample were quantitated after clean-up using both procedures with no statistically significant difference between results.

INTRODUCTION

Since recognition of the aflatoxin problem in 1960, methods recently summarized (1,2) have been developed to quantitate this toxin in contaminated products. Methods of analysis for various commodities are described in the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis (3). The CB (Contaminants Branch, FDA) Method is one such official method of analysis for aflatoxin in peanuts and pistachios (3). The procedures for determining aflatoxins in corn, soybeans and coconuts all use the CB column clean-up procedure. The CB clean-up procedure uses a silica gel column of 22 mm id × 500 mm that requires three 150-mL aliquots of eluting solvents. In 1977, Pons and Franz proposed a high pressure liquid chromatographic (HPLC) method for the quantitation of aflatoxins in cottonseed products (4). Part of the procedure involved use of a small column for extract clean-up. We have substituted this small Bio-Rad Laboratories glass Econo-Column, 10 mm id × 300 mm, hereafter termed "Pons" column, for the official one used in the CB procedure. The silica gel required for packing and the 3 elution solvents are reduced 5-fold from those required by the official procedure. The aliquot taken from the initial extraction for analysis is also reduced 5-fold. The sample size and volume of original extracting solvent are not changed, so there is no loss in sensitivity. We applied the solvent-saving modification to tests of corn, peanuts, soy-

TABLE I

Aflatoxin Content of Corn: Extracts Cleaned up with a CB or Pons Column

Sample	Aflatoxin B_1 [a] (μg/kg)		Aflatoxin B_2 [a] (μg/kg)	
	CB	Pons	CB	Pons
A	1139	863	267	155
B	658	452	128	94
C	349	355	33	35
D	103	166	12	15
E	70	74	30	31
F	4	3	ND[b]	ND

[a]Values presented are the average of duplicate 10-μL spottings from a single column extract.

[b]None detected.

beans, coconut and pistachios containing high, medium and low levels of aflatoxins.

METHODS AND MATERIALS

Corn samples were naturally contaminated with various levels of aflatoxin B_1 and B_2. Peanuts, soybeans, coconut and pistachios were prepared by mixing inoculated samples with toxin-free ones to produce the desired aflatoxin levels. Samples were cracked and adjusted to 30% moisture (5). Peanuts and soybeans were inoculated with *Aspergillus parasiticus* NRRL 2999 and coconut and pistachios with *A. flavus* SRRC 1000. To more closely simulate natural conditions, samples were not autoclaved prior to inoculation. All samples were incubated at 27 C for 7 days. After incubation, inoculated samples were dried under vacuum, ground and mixed with the noninoculated samples. Based on analyses of the inoculated samples, preparations with 3 levels of aflatoxin were formulated of peanuts, soybeans, coconut and pistachios. One naturally contaminated peanut meal and 6 corn samples of known aflatoxin content were analyzed.

A single extraction was made of each sample. Extraction procedures followed the conventional CB method, except that methylene chloride was substituted for chloroform (6). The extract was collected in a 300-mL Erlenmeyer flask, the flask was stoppered and contents mixed to insure complete homogeneity. A 50-mL aliquot was removed for the CB clean-up column and a 10-mL aliquot for the Pons column. The silica gel packing techniques were the same for both the Pons and standard size CB columns, except that the bed of sodium sulfate was eliminated for the Pons column. Ten g of silica gel was used for the CB column and 2 g for the Pons. The Pons column was topped with 3 g of sodium sulfate rather than the 15 used in the CB procedure.

Washes (150-mL) of hexane, diethyl ether and methylene chloride/methanol (97:3) were used on the CB column and 30-mL washes of each were used on the Pons column. Aflatoxins were eluted in the methylene chloride/methanol wash. A 50-mL beaker was used to collect the final eluant. Extracts from each column were evaporated on a steam bath to near dryness and quantitatively transferred in methylene chloride to 5-mL vials fitted with screw caps. This solvent was removed under a stream of nitrogen and a known volume of benzene/acetonitrile (98:2) was added to each vial prior to thin layer chromatographic (TLC) quantitation. Five, 3 and 1 mL were used for samples cleaned up through the CB column for high, medium and low aflatoxin levels, respectively, and 1, 0.6 and 0.2 mL were used for dilutions of the corresponding extracts cleaned up on the Pons column. Corn extracts with the lowest level of toxins (<5 μg/kg) cleaned up through the CB column were dissolved in 0.2 mL and 40 μL of benzene/acetonitrile (98:2) was used for extracts cleaned up by the Pons column. Two 10-μL aliquots of each extract were spotted on scored Adsorbosil-1 plates along with two 10-μL aliquots of a standard aflatoxin preparation. Extracts from the same sample cleaned up by the 2 procedures were spotted on the same TLC plate. Quantitations were made densitometrically (7).

RESULTS AND DISCUSSION

This study's one purpose was to compare the Pons column to the CB column to effect savings, especially in usage of solvents. No variation resulted from sampling because aliquots were taken from a common extraction. Aflatoxins in the methylene chloride eluant from the CB and the Pons column were quantitated on the same TLC plate, eliminating variations from plate differences.

Table I lists the results on corn samples at the 6 toxin levels. Results with peanuts are shown in Table II and those from soybeans, coconut, and pistachios are listed in Table III. Analysis of variance of all corn samples at all levels showed that there was no statistically significant difference between values obtained from the 2 columns. The coefficient of variance (CV) was 8.3%. There may have been some interaction between toxin level and column as slightly higher values were obtained from corn samples A and B (Table I) with the CB column than with the Pons column. The B_2 values followed those of B_1, showing that the columns handle the 2 toxins equally.

Analysis of variance for peanuts showed a CV of 6.0%, again indicating no statistically significant difference between values obtained from the 2 columns. Duplicate

TABLE II

Aflatoxin Content of Peanuts: Extracts Cleaned up with a CB or Pons Column[a]

Sample	Aflatoxins[b]											
	Aflatoxin B_1 (μg/kg)				Aflatoxin B_2 (μg/kg)				Aflatoxin G_1 (μg/kg)			
	CB		Pons		CB		Pons		CB		Pons	
	1	2	1	2	1	2	1	2	1	2	1	2
A	1011	1081	990	1080	726	828	685	850	315	387	323	417
B	335	336	389	335	138	130	131	96	79	81	76	44
C	133	119	129	138	66	63	70	73	27	29	35	36

[a]Duplicate 50- and 10-mL aliquots from a common extract were run through duplicate CB and Pons columns (1 and 2).

[b]Values presented are the average of duplicate 10-μL spottings from a single column extract.

TABLE III

Aflatoxin B_1 Content of Coconut, Pistachios and Soybeans: Extracts Cleaned up with a CB or Pons Column[a]

Sample	Aflatoxin B_1 (μg/kg)		Aflatoxin G_1 (μg/kg)	
	CB	Pons	CB	Pons
Soybeans				
A	1032	1004	875	830
B	533	495	202	281
C	44	55	16	23
Coconut				
A	956	1000	–	–
B	365	393	–	–
C	117	136	–	–
Pistachio				
A	954	1459	–	–
B	346	377	–	–
C	60	59	–	–

[a]Values presented are the average of duplicate 10-μL spottings from a single column extract.

analyses were run for peanut samples only (Table II). There was no statistical difference between the 2 CB columns and the 2 Pons columns. Peanut extracts that were high in toxins did not overload the Pons column. Differences were less between columns at the lower toxin levels in peanuts and corn. This is probably a function of the dilution and the spotting aliquot. Data in Table III show that the 2 columns were comparable for the clean-up of extracts from soybeans, coconut and pistachios. No statistical analyses were made of results from these 3 commodities. B_1 alone was quantitated from the coconut and pistachio samples as B_2 was not produced in sufficient quantities for quantitation. Similarly, only B_1 and G_1 were quantitated in soybeans as not much B_2 and G_2 were produced. Substrate seemed to dictate proportions of toxins formed.

Only one meal was used in this study—a sample of the original peanut meal designated as the causative agent in the death of thousands of young turkeys in the 1960 Turkey X disease—the Rossetti meal (8). There was good agreement in results from the Pons and CB columns. Values of 510 ppb of B_1 and 356 ppb of B_2 were obtained by the official CB procedure and 495 ppb B_1 and 342 ppb B_2 were obtained with the Pons column modification.

We have shown that the Pons column can be used to reduce solvent requirements without compromising sensitivity. Sensitivity with the Pons column was not decreased from that of the CB procedure because there was no reduction in sample size or in original volume of extracting solvent. Such a modification can save cost; the volume of solvents and other chemicals saved in each test is economically significant when thousands of samples are being analyzed.

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