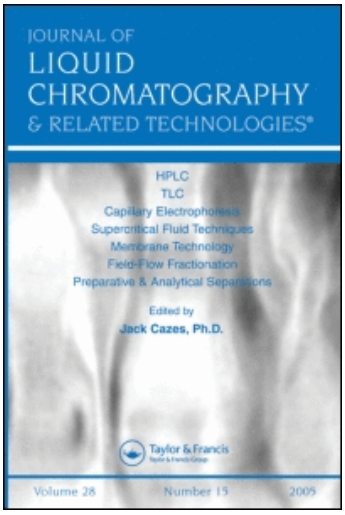

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REDUCTIVE VOLTAMMETRIC HPLC DETECTION OF AFLATOXINS: DETERMINATION OF AFLATOXIN B_1 IN FOODS

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ABSTRACT

A reductive voltammetric detector, consisting of static mercury drop electrode was investigated for possible determination of aflatoxins B_1, B_2, G_1, and G_2 by HPLC. The HPLC system consisted of a C_{18} ODS column and a mobile phase composed of 30% acetonitrile and 0.01M tetrabutylammonium bromide. The detector was operated in a differential pulse mode, set to the peak potential of -1.25 volts vs. Ag/AgCl. Under these conditions the sensitivity of the system was sufficient to determine aflatoxin B_1 present in several foods. Good agreement was obtained between this method and thin layer chromatography.

INTRODUCTION

Aflatoxins are a group of toxic metabolites produced by certain strains of the common mold, Aspergillus flavus, as well as other mold genera. They were first found in peanut meal.

The levels of aflatoxins present in a number of foods was published by Wood[1] and Setsuko, et al.[2] Animal studies have shown most of them to be highly carcinogenic and, therefore, pose a health hazard to man.

Chemically, aflatoxins B_1, B_2, G_1 and G_2 are difurancoumarin derivatives. They are intensely fluorescent compounds, and consequently many methods use this property for their detection, such as in TLC and HPLC methods.[3] Problems exist, however, with the above methods in that TLC suffers from poor precision and HPLC from low specificity. Aflatoxins have been studied by oscillographic polarography[4] and by differential pulse polarography.[5] Their reduction potentials range from -1.21 to -1.27 volts (vs. SCE). These potentials are too close to determine individual aflatoxins when present in mixtures.

The purpose of this study was to investigate the possibility of applying reductive voltammetric detection in conjunction with the separation of some important aflatoxins by HPLC. The developed method could then be used for the analysis of food products.

MATERIALS AND METHODS

Apparatus

LC Pump: Waters Model 510 (Waters Assoc., Milford, MA); Injector: Rheodyne Model 17121 (Rheodyne Inc., Catati, CA); LC Column: Zorbax ODS, 5 μm, 4.6 mm x 25 cm (DuPont Co., Wilmington, DE); Guard Column: 7 cm x 2.1 mm id., packed with 25-37 μm Co:Pell ODS (Whatman, Inc., Clifton, NJ); Polarographic Analyzer: EG & G PAR Model 264A (EG & G Princeton Applied Research, Princeton, NJ); Static Mercury Drop Electrode: EG & G Model 303 (EG & G Princeton Applied Research. Princeton, NJ); Detector: EG & G PAR Model 310, (EG & G Princeton Applied Research, Princeton, NJ)

A schematic diagram of the apparatus is shown in Figure 1.

Reagents

Aflatoxin Standards: Individual B_1, B_2, G_1, and G_2 aflatoxin standards received as dry films were separately diluted to nominal concentrations of 10 μg/mL with benzene-acetonitrile (98+2). The standard concentrations were

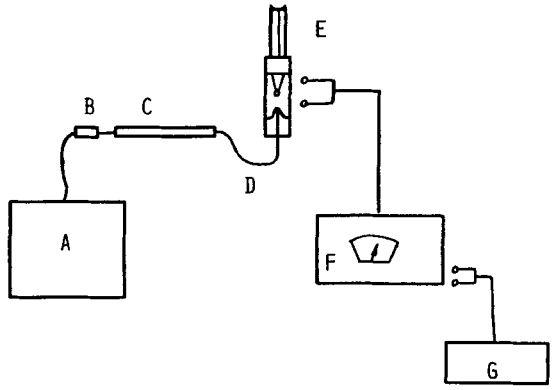

Figure 1. Schematic diagram of apparatus. A, HPLC pump; B, Guard column; C, HPLC column; D, Eluate; E, Mercury drop electrode; F, Polarograph; G, Strip chart recorder.

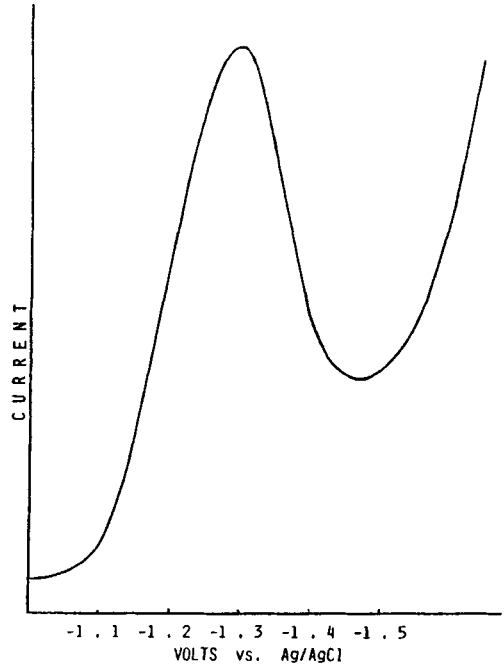

Figure 2. Differential pulse polarogram of a mixture of aflatoxins B_1, B_2, G_1, and G_2 in the mobile phase. Polarographic conditions: pulse amplitude, 100 mv; pulse time, 0.5 *s*; electrode, hanging mercury drop; sensitivity setting, 10 μA full scale.

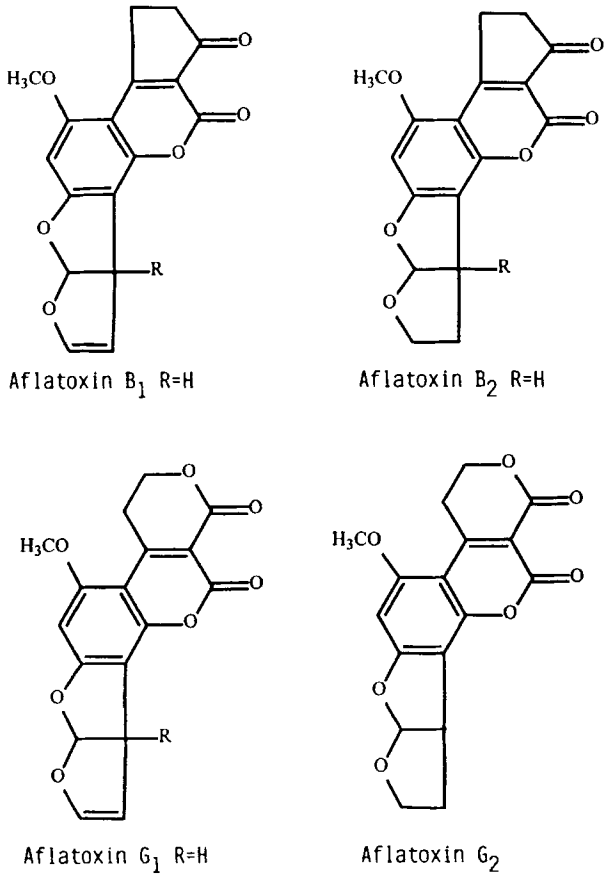

Figure 3. Structural formulas of the four aflatoxins.

determined spectrophotometrically using $K_2Cr_2O_7$ primary standard to calibrate the spectrophotometer (AOAC, 1990). For analysis by the proposed HPLC method the benzene-acetonitrile was evaporated at low heat in a current of nitrogen. The residue was then dissolved in the mobile phase.

Mobile Phase: 30% acetonitrile and 0.01M tetrabutylammonium bromide. Oxygen was removed by purging with nitrogen for twenty minutes, then nitrogen was directed above the mobile phase during analysis to prevent oxygen from re-entering.

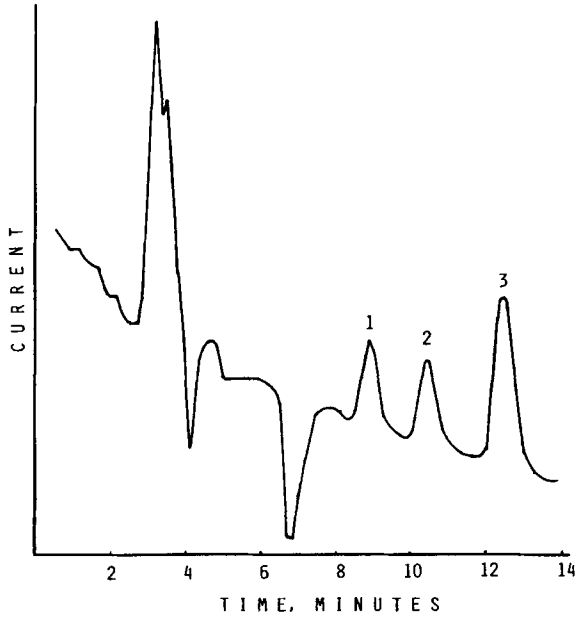

Figure 4. Chromatogram of a mixture of aflatoxins. Peak 1, G_2; Peak 2, B_2+G_1; Peak 3, B_1.

Table 1

Results of Sample Analysis by TLC and Proposed HPLC Methods

Found, ng/g[1]

	TLC				HPLC			
	B1	**B2**	**G1**	**G2**	**B1**	**B2**	**G1**	**G2**
Melon seeds	38	4	0	0	43	ND	ND	ND
Corn meal	43	5	0	0	42	ND	ND	ND
Corn flakes	54	11	0	0	48	ND	ND	ND
Mixed nuts	0	0	0	0	ND	ND	ND	ND

[1] Single determinations.
ND: Not detected.

Samples

Melon seeds, corn meal, corn flakes and mixed nuts were obtained as samples from the importers of these products.

Preparation of Samples

Samples were prepared and analyzed according to the official CB method.[3] The extracts were contained in vials in small volumes of benzene-acetonitrile (98+2) and were stored in a freezer. Prior to the analysis by the proposed method, the solvent was evaporated at low temperature in a current of nitrogen. The residues were then dissolved in 0.5 mL of the mobile phase for analysis by the proposed HPLC method.

Procedures

Polarography: Differential pulse polarograms were run on standards dissolved in the mobile phase in order to determine the potential setting required for the detection in HPLC effluents.

HPLC conditions: Flow rate, 1 mL/min.; potential, -1.25 volts vs. Ag/AgCl; electrode: hanging mercury drop; mode, differential pulse; modulation amplitude: 100 mv; volumes injected: 200 μL.

RESULTS AND DISCUSSION

Prior to HPLC analysis, it was necessary to study the voltammetric behavior of the compounds in question. A mixture of aflatoxins B_1, B_2, G_1 and G_2 dissolved in the mobile phase of 30% acetonitrile-0.01M tetrabutylammonium bromide and polarographed in a differential pulse mode gave a single peak at -1.25 volts vs. Ag/AgCl (Fig. 2). This was to be expected due to the similarity of their chemical structures, Wilson et al.,[6] (Fig. 3). Solutions of individual aflatoxins and their mixtures were injected into the proposed HPLC system in order to obtain their retention times, separation and detection parameters. A chromatogram of a mixture of the four aflatoxins is shown in Fig. 4. Peaks 1 and 3 are aflatoxins G_2 and B_1, respectively, while peak 2 represents unseparated aflatoxins B_2 and G_1. No attempt was made to separate these aflatoxins at the present time since the primary objective of this study was to determine the feasibility of voltammetric detection.

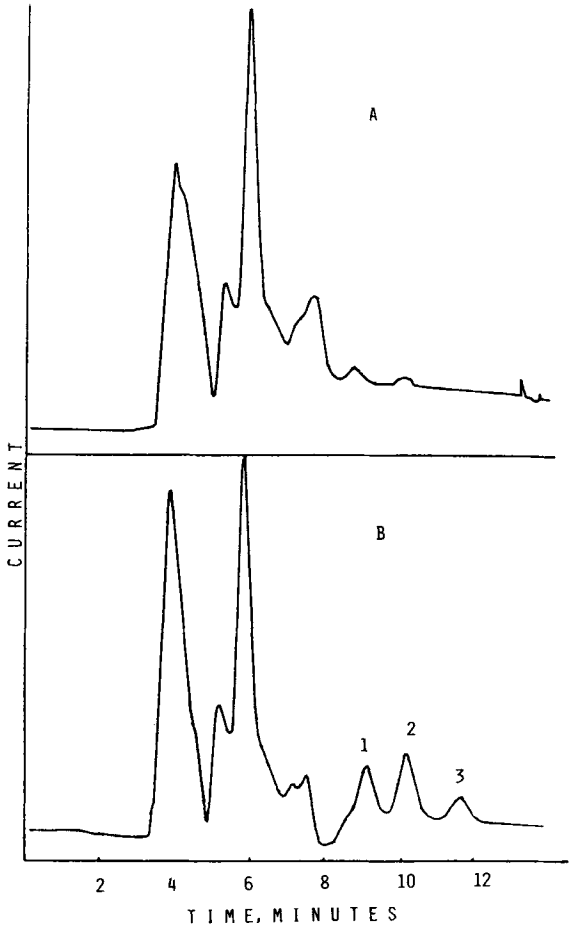

Figure 5. Chromatogram of a mixed nut sample, A; and B, mixed nut sample spiked with 20 ng/g B_1, B_2, G_1, and G_2. Peak 1, G_2; Peak 2, B_2+G_1; Peak 3, B_1.

In order to determine the practicality of the technique, several different sample extracts and spikes were injected into the proposed chromatographic system. Typical chromatograms of a nut sample and spike are shown in Fig. 5, while a chromatogram of a corn sample is shown in Fig. 6. Since the retention times were somewhat affected by the sample matrix, identification of the peaks was made by standard additions. In order to compare the proposed method with the official TLC method, experiments were conducted on the food samples.

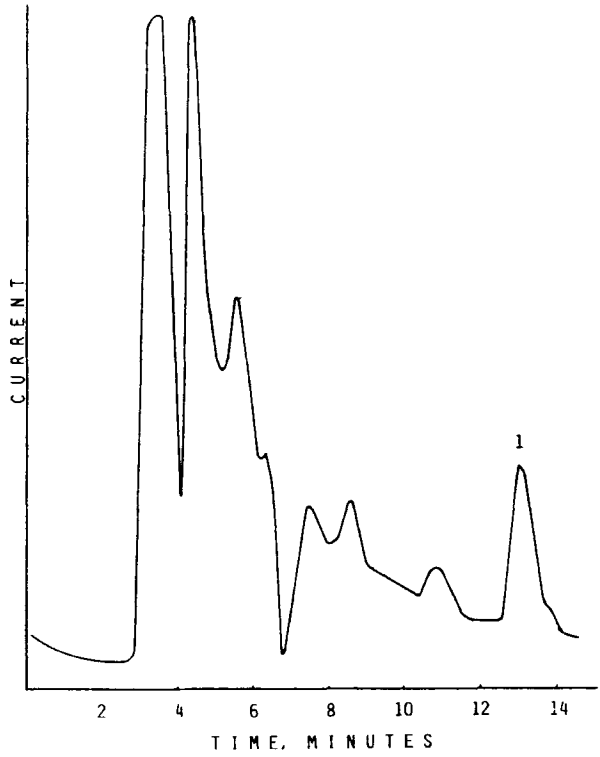

Figure 6. Chromatogram of corn sample. Peak 1, aflatoxin B_1.

The results of this comparative test are given in Table 1. The sensitivity of the proposed HPLC method was sufficient only to detect and quantitate aflatoxin B_1; the detection limit was 2.5 ng/g. The agreement for this aflatoxin between the methods, however, is very good.

CONCLUSION

The method as tested was able to determine aflatoxin B_1 which may occur in foods. It should be useful for confirmation of results found by the TLC method. In order to detect other aflatoxins that may be present at very low levels (<ppb to low ppb) it will be necessary to modify the HPLC conditions and enhance the sensitivity of the system.

A possible approach may be to increase the sample size and to switch to a gold/mercury electrode connected to a polarographic analyzer capable of differential pulse mode of detection. Work is continuing in this area.

ACKNOWLEDGEMENT

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