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Note

High-performance liquid chromatographic determination of ochratoxin A in artificially spiked cocoa beans

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Mycotoxins are toxic mold metabolites produced during the growth of molds in various foods and feed grains. One of these mycotoxin groups is that of ochratoxin. The ochratoxins are produced by *Aspergillus ochraceus* and *Penicillium viridicatum*[1]. These molds are found in soil and in various foods and feeds[2–4]. Ochratoxin A is the 7-carboxy-5-chloro-8-hydroxyl-3,4-dihydro-3-R-methylisocoumarine amide of L-β-phenylalanine and is said to be the one produced in the largest amount[5]. There are other ochratoxins and esters, but the esters have only been produced in laboratory environments[5] and due to the relative intensity of ochratoxin A, the reasons for choosing it are evident.

Ochratoxin A has been reported to occur in a wide variety of foods including wheat, barley, coffee, beans and rye at levels ranging from < 10 to 28 mg/kg[6]. As a commodity, cocoa has been surveyed[6] but the methods exhibited low recoveries, 40–60%, and lower limits of 50–100 μg/kg. The purpose of this method was to extend the lower limits that have been reported, improve recoveries and evaluate methods to confirm the identity of a possible ochratoxin A peak. High-performance liquid chromatography (HPLC) has been used in the analysis of various products for ochratoxin A[1–4]. This method reports the use of HPLC for the analysis of ochratoxin A in cocoa beans.

MATERIALS AND METHODS

Ochratoxin A

Ochratoxin was purchased from Sigma (St. Louis, MO, U.S.A.) and kept frozen (−20°C) until needed. When needed the ochratoxin was dissolved in chloroform to make a stock solution of 0.1 mg/ml. For HPLC usage, the stock solution was evaporated to dryness and brought up in HPLC mobile phase for a final concentration of 0.02 μg/μl.

HPLC

The HPLC system of an M6000A solvent delivery system (Waters Assoc.), U6K injector (Waters Assoc.), a Gilson Spectra-Glo Filter fluorometer [λ (excitation) 365 nm; λ (emission) 421 nm], and a Model 440 UV detector (Waters Assoc.). The mobile phase was methanol–0.1% phosphoric acid (1:1) containing 0.005 *M* hep-

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tanesulfonic acid (pH 3.5 ± 0.1). The flow-rate was 1.0 ml/min and the HPLC column was Spherisorb ODS (30 cm × 3.9 mm I.D.).

Extraction procedure

The procedure used was an adaptation of the recognized methods for the isolation of ochratoxin A[7,12]. A 50-g sample of cocoa beans was blended with 200 ml of extracting solvent for 3 min of high speed in a Waring blender. The extracting solvent used is chloroform–methanol–hexane (8:2:1). The resulting extract was filtered through a Buchner funnel and the blender cup rinsed with an additional 50 ml of solvent. The entire filtrate representing 50 g was transferred to a separatory funnel and 100 ml of 0.1 *M* sodium bicarbonate were added. The funnel was shaken for 1 min and the bicarbonate layer was withdrawn and saved. The resulting organic phase was reextracted with an additional 100 ml of sodium bicarbonate. These two bicarbonate layers were combined and the organic layer discarded. The aqueous layer was adjusted to pH 3.0 ± 0.1 with hydrochloric acid and extracted with three 50-ml portions of chloroform. The chloroform fractions are collected over 5.0 g Na_2SO_4. The dry chloroform fraction was rotovaped using low heat (30°C) and a vacuum and dissolved in HPLC mobile phase for subsequent HPLC analysis. For HPLC analysis 50 μl were injected. If a peak occurs in the retention window for ochratoxin A, further steps are taken to insure the identity of the peak. The steps included absorbance

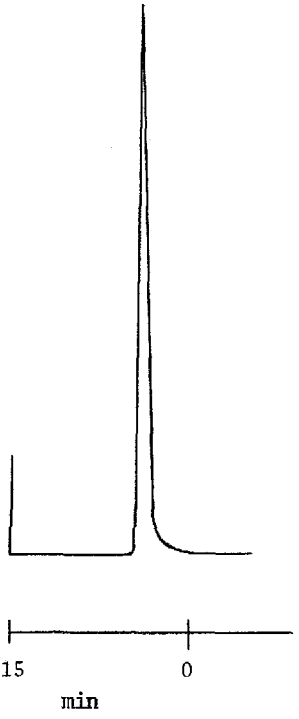

Fig. 1. Chromatogram of ochratoxin A standard. Column: Spherisorb ODS (30 cm × 3.9 mm I.D.). Mobile phase: methanol–1% phosphoric acid (1:1) + 0.005 *M* heptanesulfonic acid. Detection: fluorometer; λ (excitation) 365 nm, λ (emission) 421 nm.

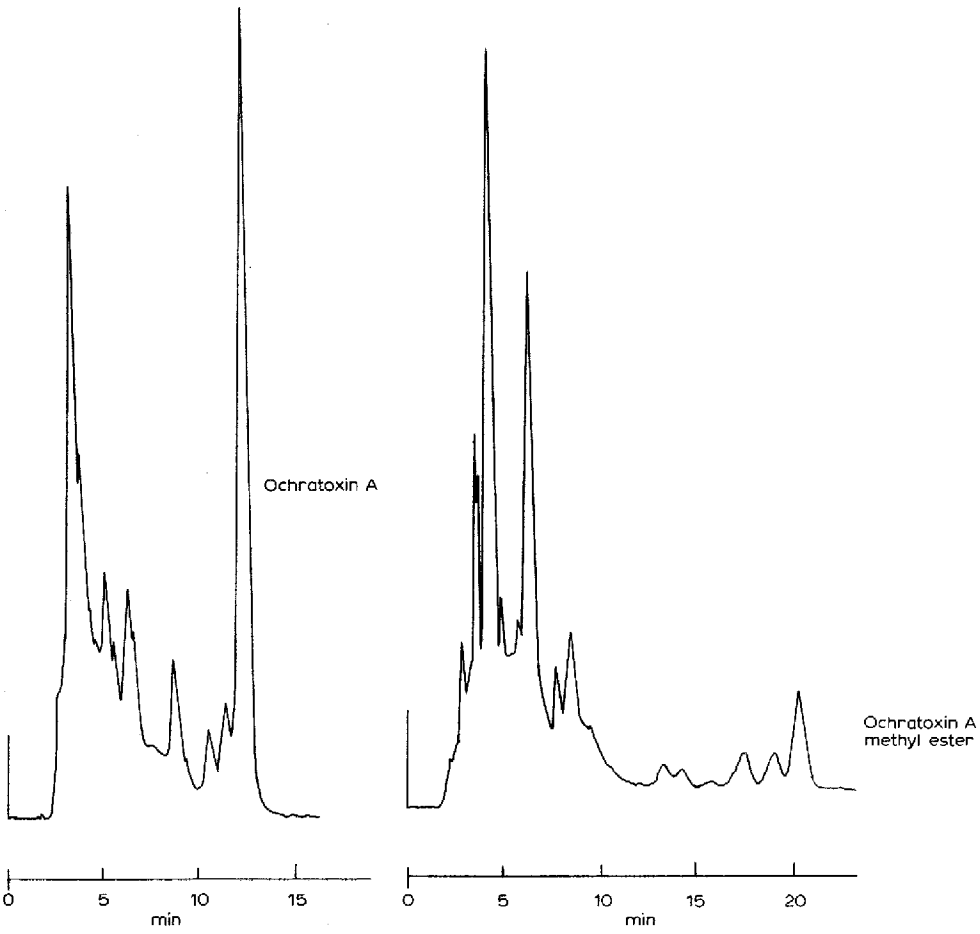

Fig. 2. Chromatogram of artificially contaminated cocoa bean. Conditions as in Fig. 1.

Fig. 3. Chromatogram of artificially contaminated cocoa bean derivative. Conditions as in Fig. 1.

ratioing at the wavelengths of 254 nm and 365 nm. The absorbance ratio (254 nm/365 nm) for ochratoxin A standard and suspect peaks were 1.25 and 1.26 respectively. This agreement gives confirmatory evidence for the identity of a suspect peak as ochratoxin A[8]. Figs. 1 and 2 show standard and artificially contaminated sample, respectively.

Derivatization

The sample containing a suspect peak is used for further studies by the preparation of the methyl ester[9,11]. The disappearance of the suspect peak and the appearance of a peak of similar size as the same retention time as the methyl ester is further confirmatory information[10,13]. Fig. 3 shows ochratoxin A methyl ester in artificially contaminated cocoa beans.

RESULTS AND DISCUSSION

The analytical procedure was evaluated by conducting accuracy and precision studies. Lower detection limits are 10 μg/kg. Table I outlines precision study while Table II outlines recovery studies.

TABLE I

PRECISION STUDIES

Sample	*n*	*Concn (μg)*	*C.V. (%)*
Standard	5	0.1/inj.	2.1
Sample	5	20/kg	3.4

TABLE II

RECOVERY STUDIES

Added (mg/kg)	*Recovered (μg/kg)*	*Recovery (%)*
10	10.1	101
20	19.7	98.5
50	46.4	92.8

The results show good recoveries of added ochratoxin and good method precision at the levels examined. This work adds a method that is now applicable to cocoa beans in the area of mycotoxin analysis. It provides the analyst with an accurate, precise method at the μg/kg level.

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REFERENCES

1 D. C. Hunt, L. A. Phillip and N. T. Crosby, *Analyst (London)*, 104 (1981) 1171–1175.
2 E. Josefsson and T. Moller, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 1165–1168.
3 B. G. Osborne, *J. Sci. Food Agric.*, 30 (1979) 1065–1070.
4 D. C. Hunt, A. T. Bourdon, P. J. Wild and N. T. Crosby, *J. Sci. Food Agric.*, 29 (1978) 234–238.
5 P. M. Scott, W. van Walbeek, B. Kennedy and D. Anyeti, *J. Agr. Food Chem.*, 20 (1972) 1103.
6 W. van Walbeek, *Can. Inst. Food Technol. J.*, 6 (1973) 96.
7 O. L. Shotwell, C. W. Hesseltine, E. E. Vandegraft and M. L. Goulden, *J. Amer. Assoc. Cereal Chem.*, 16 (1971) 266–270.
8 R. Yost, J. Stoveken and W. McLean, *J. Chromatogr.*, 134 (1977) 73.
9 S. J. Nesheim, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 975.
10 S. Nesheim, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 822–826.
11 S. Nesheim, in J. V. Rodricks (Editor), *Mycotoxins and Other Food Problems*, American Chemical Society, Washington, DC, 1976, p. 277.
12 L. J. Vorster, *Analyst (London)*, 94 (1969) 136.
13 D. C. Hunt, B. R. McConnie and N. T. Crosby, *Analyst (London)*, 105 (1980) 89.