Determination of Aflatoxin M_1 in Raw Milk: A Modified Jacobson, Harmeyer and Wiseman Method

ABSTRACT

A modification of the method of Jacobson, Harmeyer and Wiseman for the determination of aflatoxin M_1 in raw milk is made, eliminating the column clean-up which gives clean extracts, and good recoveries comparable to the parent procedure. Recoveries within the limits of visual comparison are obtained from spiked raw milk samples containing 0.3 ppb, and clean-up is sufficient to quantitize levels of 0.2 ppb in naturally contaminated raw milk. Deproteinized milk (80% methanol extracts) is adjusted to a 50% methanol concentration with 4% sodium chloride solution and lipids removed by extraction with hexane. Aflatoxin M_1 is extracted from the 50% methanol and sodium chloride phase with chloroform. The chloroform extract containing aflatoxin M_1 is then washed with 4% sodium chloride solution dried over anhydrous sodium sulfate and evaporated on a steam bath under nitrogen. The dry residue is then dissolved in chloroform and spotted on silica gel thin layer plate and developed according to the procedure of Stubblefield, Shotwell and Shannon.

INTRODUCTION

A modification of the method of Jacobson et al. (1) for the determination of aflatoxin M in raw milk is reported. This modification eliminates many of the interferences excountered in the original method, resulting in cleaner extracts with greater sensitivity of the analytical method.

EXPERIMENTAL PROCEDURE

Reagents and Materials

All reagents and solvents are ACS grade. Diatomaceous earth is Johns-Manville Celite 545, acid-washed, analytical grade. For thin layer chromatography, (TLC) silica gel plates are prepared using Adsorbosil-1 and developed in isopropyl alcohol-acetone-chloroform 5:10:85 v/v/v, according to the procedure of Stubblefield et al. (2).

SAMPLE EXTRACTION AND PURIFICATION

A 75 ml sample of raw milk is blended with 300 ml methanol for 2 min in a 1 liter blender at high speed, 25 celite is added and the mixture blended at low speed for an additional 30 sec. The mixture is then filtered through a preformed bed of Celite 545 by packing a 25 slurry of Celite 545 in 150 ml methanol with vacuum on a coarse fritted glass Buchner funnel. The precipitated protein and celite bed is then pressed and washed with 100 ml methanol. The combined filtrate and washings are transferred to a 1 liter separatory funnel and adjusted to a 50% methanol concentration by the addition of 4% sodium chloride solution. The alcoholic extract is extracted with three successive 200 ml volumes of hexane followed by three successive extractions with 100 ml chloroform to extract the aflatoxin M_1. The combined chloroform extracts are then washed with 300 ml of 4% sodium chloride solution. The washed chloroform extract is drained through a 2 in. column of anhydrous granular sodium sulfate contained in a Butt tube into a 600 ml beaker. The extract is evaporated to near dryness over a blanket of nitrogen, transferred quantitatively to a 1 dram vial and evaporated to dryness under a stream of nitrogen. The dry extract is then dissolved in the appropriate volume of chloroform and spotted on silica gel plates and developed in a Thomas-Mitchell Stainless Steel Tank according to procedure of Stubblefield et al. (2). The concentration of aflatoxin M_1 is estimated by visual comparison of the florescence of standard and test spots under UV light.

TABLE I

Recovery of Aflatoxin M_1 Added to Clean Raw Milk

Sample	M_1 Added, ppb	M_1 Recovered, ppb	Recovery, %
1	1.0	0.95	95
2	1.0	1.00	100
3	0.5	0.45	90
4	0.5	0.50	100
5	0.3	0.24	80
6	0.3	0.24	80

RESULTS AND DISCUSSION

This method is essentially that of Jacobson et al., with modification of the use of a preformed bed of Celite 545 on a coarse fritted Buchner funnel in the filtration of the initial methanol extract of the milk. This preformed celite bed yields clear filtrates with less interferences than the parent method.

Other modifications are made in volumes of hexane washes and chloroform extractions, along with the adaptions of the TLC methods of Stubblefield et al. With this TLC solvent system aflatoxin M_1 migrates to an RF position well above the yellow florescing spots occurring naturally in chloroform extracts of raw milk. The extraction and purification methods of Jacobson et al, modified slightly, and elimination of the use of Celite column clean-up provides sufficient removal of lipids and extraneous interfering materials to provide sufficiently clean extracts to allow determination of aflatoxin M_1 in raw milk at levels of 0.1 ppb with confidence.

Recovery of aflatoxin M_1 in spiked clean raw milk samples gave the results shown in Table I. These recovery percentages were comparable to those of Jacobson et al.

JOHN D. MCKINNEY
Ranchers Cotton Oil
Fresno, California 93708

ACKNOWLEDGMENT

R.O. Stubblefield, Northern Regional Research Laboratory, Peoria, Illinois, provided aflatoxin M_1 reference standards.

REFERENCES

1. Jacobson, W.C., W.C. Harmeyer and H.G. Wiseman, J. Dairy Sci. 54:21 (1972).
2. Stubblefield, R.o., O.L. Shotwell and G.M. Shannon, 85th Annual Meeting Association of Official Analytical Chemists, Washington, D.C., October 1971, Abstract No. 203; J. Ass. Offic. Anal. Chem., in press.

[Received April 7, 1972]