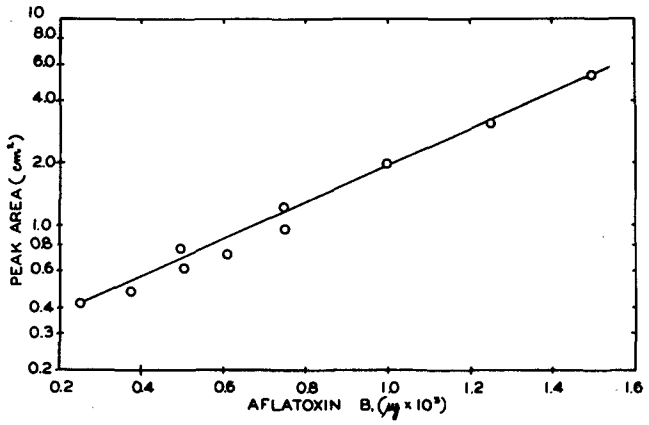

FIG. 2. Relationship of peak area to concentration of aflatoxin B_1.

the stage with the stationary phase down and the response selector switch set at 5. At this setting, the area under the recorded response is a logarithmic function of the light intensity. The zero line is adjusted with the dark point control and sensitivity control on the multiplier photometer unit. To check the sample for interfering material, the sample is spotted and developed by the usual method (3,4). The plate is then positioned so that the aflatoxin B_1 spot is in the light path. The sensitivity is then adjusted using the full light control to give the desired pen travel. The plate is scanned from the origin to the solvent front for each sample and for an aflatoxin B_1 standard.

For quantitative analysis several standard solutions were prepared using pure aflatoxin B_1 and the ultraviolet spectrophotometric extinction coefficients reported by Asao et al. (1). These standards along with 1, 2 and 4 μl portions of sample extract are spotted and the plate developed by the usual solvent system (3,4). After development the plate is placed so that the direction of scan is perpendicular to the direction of development. The plate is positioned over the aflatoxin B_1 standard of highest concentration and the recorder response adjusted with the full light control. The plate is then scanned by means of the motor drive on the TLC stage and the recorder (Fig. 1). The peak areas are determined by $A = \frac{1}{2}$ Base $\times$ Height and areas for standards are plotted against aflatoxin B_1 concentration on semilog paper (Fig. 2). The concentration of the sample is then determined by interpolation from this plot.

Standard deviation may be calculated from the three sample aflatoxin B_1 areas. An internal standard may also be applied to the sample spots before development as an additional check on the method. After development and fluorodensitometry the added aflatoxin may be subtracted from the interpolated sample value.

TABLE I

Comparison of Visual and Fluorodensitometry Methods for the Determination of Aflatoxin B_1

Cottonseed No.	Engebrech, Ayres and Sinnhuber procedure (3)		Pons and Goldblatt procedure (4)	
	Visual	Fluorodensitometry	Visual	Fluorodensitometry
1	190 ppb	182 ± 12 ppb	150 ppb	178 ± 15 ppb
2	80	82 ± 6	110	90 ± 9
3	150	146 ± 19	150	153 ± 14
4	19	22 ± 3	20	23 ± 5

The results obtained from four aflatoxin-containing cottonseed meal samples are shown in Table I.

For both the visual and fluorodensitometry techniques, 10 standards, 3 sample and 3 sample plus internal standard spots were compared. The standard concentrations ranged from 0.25 to 1.5×10^{-3} (μg aflatoxin B_1) per spot and the internal standard used was 0.25×10^{-3} (μg aflatoxin B_1). This scan is illustrated in Figure 1.

The sensitivity of the method is limited by the presence of interfering materials and their separation from aflatoxins on the TLC plate. Pure aflatoxin in amounts as low as 8×10^{-5} μg on a TLC may be quantified. With lesser amounts, instrument noise becomes a problem and the fluorescence background on the plates interferes with the symmetry of the peaks on the scan.

J. L. AYRES
R. O. SINNHUBER
Department of Food Science and Technology
Oregon State University
Corvallis, Oregon

ACKNOWLEDGMENTS

Gift of aflatoxin B_1 from G. N. Wogan of Massachusetts Institute of Technology. This investigation was supported in part by USPHS Grant CA-06285 and in part by the Bureau of Commercial Fisheries.

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[Received February 24, 1966]

• *Addendum*

JAOCS 43, 168–170 (1966) L. N. Norcia and Barbara E. Rosenthal: "Sterol Content of Some Plant Oils; Further Observations on Fast-Reacting Sterols."

FIG. 1. Absorption spectra determined for 0.4 mg sterol/s per tube; color development at 6 ml total volume for 30 minutes, 25C; 1 cm light-path.