Waters
Lab Highlights
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EXCELLENT SENSITIVITY FOR AFLATOXINS ON RADIAL-PAK™ NOVA-PAK™ C_{18}

Until the early 1960's it was thought that mold growth on foods was harmless. At that time it was found that some strains of the fungus Aspergillus flavus produced compounds which caused cancer in some animal species and which could be lethal if ingested in sufficiently large quantities. These compounds were called AFLATOXINS. Since that time, a number of other fungal toxins have been found and are given the general term MYCOTOXINS.

Although there is significant interest in all these mycotoxins, aflatoxins are still the focus of much attention, partly because they are indicators of general fungal infection of foods and also because many countries have introduced legislation forbidding the sale of foods containing aflatoxins above certain levels. The regulations vary from place to place but, as a general rule where regulations exist, the maximum level of aflatoxins allowed in foods does not exceed 20 μg/Kg (ppb).

TLC is still used widely for the estimation of aflatoxins but LC is often used where the advantages of increased sample throughput with unattended operation are desired. If accurate quantitation is required, the TLC method requires the use of a densitometer, which is an expensive piece of equipment, and LC becomes an economically viable alternative.

Much early work on aflatoxin separation was done on normal-phase columns following the methods of Walter Pans, but more recent methods have tended towards reverse-phase separations. Aflatoxins B1 and G1 do not fluoresce strongly in reverse-phase solvents but strongly-fluorescing water adducts are formed readily by reacting these aflatoxins to form aflatoxin B 2a and G 2a, so that all the aflatoxins produce approximately equivalent fluorescence response.

A special combination of filters and lamp are available for the Waters Model 420 AC Fluorescence Detector which give a sensitivity for aflatoxins which exceeds that available from the instruments of many other manufacturers.

This high sensitivity is demonstrated on a Radial-PAK™ NOVA-PAK™ C_{18} in Figure 1 (Trace A). An injection of 2 ng of each aflatoxin produced UV peaks which are barely discernable from baseline disturbances.

Under the same conditions, the Model 420 AC produced signals (Trace B) about 1/2 f.s.d. at a gain of only X2 (with a possible maximum of X 128). Thus, the Model 420 AC has about 50 TIMES THE SENSITIVITY OF UV DETECTION for these compounds when fitted with the optimum lamp and filters. To obtain this high sensitivity it is necessary to use the special lamps and filters specified below.

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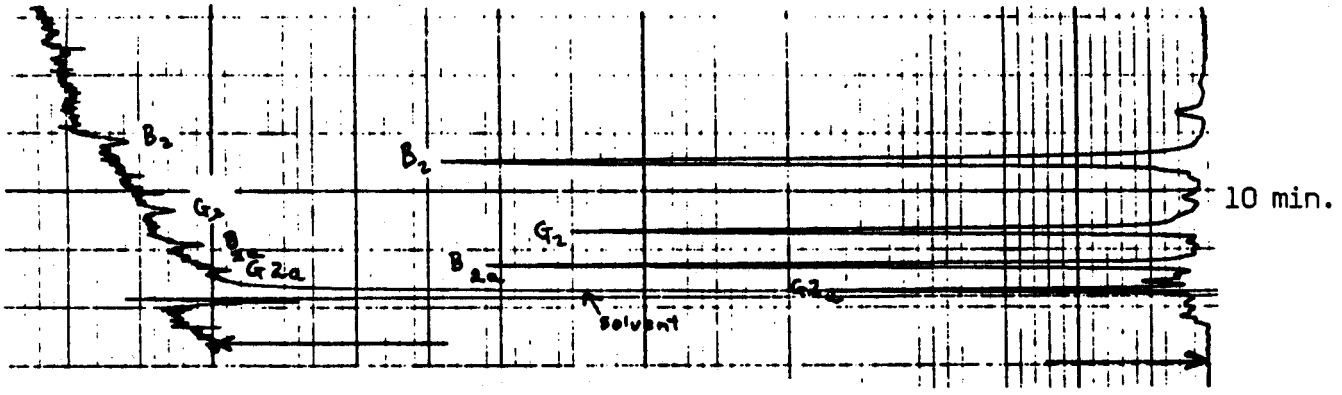
The chromatogram in Figure 1 was obtained on a NOVA-PAK™ cartridge and shows excellent resolution.

FIGURE 1

2 ng of each aflatoxin injected

Trace A
UV Detection

Trace B
Fluorescence Detection



TRACE A
Model 440 Detector
0.005 AUFS
365 nm

TRACE B
Model 420 AC Detector
Ex: 360 nm filter P/N 78154
Em: 425 nm filter P/N 78155
"Aflatoxin" lamp P/N 78409
Span - Full
Gain - X 2

Column: NOVA-PAK™ cartridge, 8mm I.D. in Z-Module™ or RCM-100®

Mobile Phase: 20% Methanol
20% Glacial Acetic Acid
60% Water
pH to 4.0 with conc. NaOH.

Flow Rate: 2 ml/min.

This mobile phase is rather viscous but produces an excellent separation and good peak symmetry.

For further information the following references are recommended:

1. Waters Status Report #19.
2. Betina, V. (Ed.). "Mycotoxins. Production, Isolation, Separation and Purification." Elsevier, 1984.