Chromatographic Analysis of Mycotoxins on Thin-Layers of Rice Starch

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Key Words
Mycotoxins
TLC
Rice starch as adsorbent

Summary
Chromatographic separation and UV detection of twelve mycotoxins on thin-layers of rice starch are presented. The effect of solvent systems on the fluorescence of mycotoxins and the mechanism of the chromatographic separation are described.

Introduction
Since the discovery of aflatoxins [1] much time and effort have been done to develop sensitive and reliable analytical procedures for the determination of mycotoxins in food and feedstock.

There are three official A. O. A. C. methods for the analysis of aflatoxins. The so-called Celite method [2] is using a Celite 545 column; in the CB method [3, 4] purification of the aflatoxins takes place on a silica gel column; finally, in the third method a separatory funnel [5] is used instead of the Celite column. Combinations of these methods are also known for the determination of a limited number of mycotoxins. A screening procedure for the determination of aflatoxins and zearalenone was developed by Eppley [4], while Vorster [6] established a method, similar to Eppley's, for the separation of sterigmatocystin, ochratoxins and aflatoxins. Scott and Somers [7] described a procedure, for patulin analysis investigating the stability and carcinogenic effect of patulin in fruit products.

Even although methods are available for the most important mold toxic metabolites of mycotoxins (aflatoxins, ochratoxins sterigmatocystin, zearalenone and patulin) they continuously change in their basic steps (e.g. sampling, defatting, extraction, purification and quantitative evaluation) [1–7].

Although, the determination of mycotoxins is usually carried out by thin-layer chromatography using various types of silica gels, in the present study rice starch was examined as the adsorbent. As shown earlier, this material has excellent separation characteristics for some other classes of organic compounds [8–10].

Experimental
Samples
The following mycotoxin standards were provided as gift by the laboratories listed: aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, aflatoxin M2, zearalenone, zearalenol, ochratoxin A, ochratoxin B, ochratoxin B ester and ochratoxin C. The gifts were obtained from: the Department of Food Sciences of the University of Alberta, Edmonton, Alberta, Canada; the U.S. Food and Drug Administration 200 C Street, S.W. Washington, D.C. 20204, USA; Rijks institut voor de Volkgenezheid, Antonie van Leewenhoeklaan, Nederland; the Department of Plant Pathology University of Minnesota St. Paul, Minn. 55108, USA; D.I.V. I.M.C. Chemical Group INC., Terre Haute, Indiana 4780, USA; Bundesanstalt für Fleischforschung, D-8650 Kulmbach, FRG, Calbiochem AG, Loewengraben 14, CH-6000 Luzern 5, Switzerland.

The concentration of the mycotoxin solutions applied on the chromatoplates were as follows:
- chloroform solution: aflatoxin B1 0.5 ng/cm², aflatoxin B2 1.5 ng/cm², aflatoxin G1 0.5 ng/cm², aflatoxin G2 1.5 ng/cm², aflatoxin M1 2.0 ng/cm², aflatoxin M2 2.0 ng/cm², ochratoxin A 20 ng/cm², ochratoxin B 20 ng/cm², ochratoxin B ester 20 ng/cm², ochratoxin C 20 ng/cm²;
- benzene solution: zearalenone 0.8 mg/cm² and zearalenol 0.8 mg/cm³.

Preparation of the Plates
Chromatoplates (20 X 20 cm) were prepared for TLC analysis by mixing 16 g of rice starch (Carlo Erba, Milano, Italy) and 2 g CaSO₄ with 22 cm³ of 95 % ethanol and 22 cm³ of distilled water in a Waring blender for 30 s. The slurry was spread on the plates forming a 0.250 mm thick layer with the equipment of Desaga. The plates were dried at room temperature for 24 h and stored in a desiccating cabinet.

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Solvent Systems

I: 20 cm$^3$ benzene + 0.15 cm$^3$ formic acid (98–100 %)
II: 20 cm$^3$ toluene + 0.15 cm$^3$ formic acid (98–100 %)
III: 20 cm$^3$ xylene + 0.15 cm$^3$ formic acid (98–100 %).

All solvents used were of analytical grade quality.

Plate Development

The standard solutions were spotted on the plates both alone and as mixtures using a glass microcapillary; 5 mm$^3$ of mycotoxin was spotted on the plate. The plate was developed by the ascending technique in a tank at room temperature, then dried and finally inspected with a high-intensity ultraviolet lamp.

Detection of Mycotoxins

Detection was carried out by UV light at the wavelengths of 366 nm and 254 nm, in order, to locate the zones of fluorescence.

Results and Discussion

By applying solvent systems I–III it was possible to successfully separate the mixture of aflatoxins, ochratoxins, zearalenone and zearalenol (appearing as two separate fluorescent spots) on the thin-layer of rice starch. Table I lists the $R_f$ values measured.

The strongest fluorescence and the best separation of the zones were obtained using solvent system II, while in solvent systems I and III less sample components could be separated. The fluorescence became weaker with increasing developing time. Fig. 1 shows a typical separation on the rice starch plate, using solvent system II.

Survey of the literature [9–11] confirmed the necessity of having a small amount of a polar component (water) in the non-polar solvent systems during the chromatographic analysis of various organic compounds on starch layer.

In order to obtain a satisfactory separation of a mycotoxin mixture on rice starch layer, small amounts of formic acid were added as the necessary polar component, to benzene, toluene and xylene used as the developing solvent. Thus, the given amount of formic acid increased the selective separation of the mycotoxin mixtures in benzene, toluene and xylene and the best was obtained with toluene (solvent system II).

Besides a predominantly partition mechanism, adsorption also contributed in a limited way to the separation of mycotoxins on starch layer. This may be explained by mycotoxin ability to form the hydrogen bonding with various macromolecules, especially with maltose units, very rich in hydrogen atoms (see Fig. 2).

### Table I. $R_f$ values of mycotoxins in various solvent systems on rice starch thin-layer

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Aflatoxin B$_1$</td>
<td>0.377</td>
</tr>
<tr>
<td>Aflatoxin B$_2$</td>
<td>0.305</td>
</tr>
<tr>
<td>Aflatoxin G$_1$</td>
<td>0.179</td>
</tr>
<tr>
<td>Aflatoxin G$_2$</td>
<td>0.126</td>
</tr>
<tr>
<td>Aflatoxin M$_1$</td>
<td>0.024</td>
</tr>
<tr>
<td>Aflatoxin M$_2$</td>
<td>0.024</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>0.868</td>
</tr>
<tr>
<td>Zearalenol (h)*</td>
<td>0.712</td>
</tr>
<tr>
<td>Zearalenol (l)*</td>
<td>0.629</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>0.443</td>
</tr>
<tr>
<td>Ochratoxin B</td>
<td>0.263</td>
</tr>
<tr>
<td>Ochratoxin B-ester</td>
<td>0.970</td>
</tr>
<tr>
<td>Ochratoxin C</td>
<td>0.970</td>
</tr>
</tbody>
</table>

* Higher (h) and lower (l) position of zearalenol, as they were two compounds.