Liquid chromatographic determination of fumonisins B$_1$ and B$_2$ in corn samples after reusable immunoaffinity column clean-up

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Abstract: The possibility of the liquid chromatographic determination of fumonisins B$_1$ (FB$_1$) and B$_2$ (FB$_2$) in corn samples with a reused immunoaffinity column (IMA) for the clean-up of the samples was investigated. After optimization of the chromatographic determination of FB$_1$ and FB$_2$ derivatized with o-phthaldialdehyde-2-mercaptoethanol, the efficiency of the clean-up of spiked corn extracts with reuse of the IMA columns was studied, both with and without column regeneration. It was found that the IMA column, designated for single-use only, can be used at least five times without regeneration and additional five times after regeneration. Regeneration consists of leaving the phosphate buffer saline solution on the column for one day at 4 °C. The efficiency of the columns was tested by determining the recovery of FB$_1$ and FB$_2$, as well as the reproducibility of the determinations. The mean recoveries of FB$_1$ and FB$_2$ from corn spiked with FB$_1$ at 1.0 mg/kg and with FB$_2$ at 0.5 mg/kg (on the basis of 10 measurements) were 88.7% (RSD 10.2%) and 90.5% (RSD 6.1%), respectively.

Keywords: mycotoxins, fumonisins, liquid chromatography, immunoaffinity column, regeneration, corn analysis.

INTRODUCTION

Fumonisins, secondary metabolites of fungi from the genera Fusarium, are mycotoxins, of importance to human and animal health. They are the most frequently found in corn and corn-based products worldwide. Fumonisins have experimentally been shown to be a causative agent of equine leukoencephalomalacia and porcine pulmonary edema syndrome, and to produce liver cancer in rats. Acute fumonisin toxicity in humans has not been confirmed, but the presence of fumonisins in corn was statistically associated with the high incidence of esophageal can-

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cer in people from South Africa\textsuperscript{3} and China.\textsuperscript{4} The International Agency of Research of Cancer has classified \textit{Fusarium moniliforme} toxins as potential carcinogens for humans (class 2B carcinogens), similar to ochratoxin A.\textsuperscript{5} An official tolerance value for dry corn products (1 µg/g) has been issued only in Switzerland, while only recommendations have been issued in the United States and France.\textsuperscript{6} A tolerance level for fumonisins in feed and groceries has not yet been set in Serbia and Montenegro. There are also no available data about their presence in our country.

![Chemical structures of fumonisins B1, B2, B3 and B4.](image)

Structurally, fumonisins are polar organic compounds with a long hydrocarbon chain. According to their structure, there are four series: A, B, C and P. Most attention has been devoted to toxins from the B series, since they are the most toxic. These toxins are diesters of propane-1,2,3-tricarboxylic acid and 2-amino-12,16-dimethylpolyhydroxyeicosanes (Fig. 1).\textsuperscript{1,7} Many analytical procedures have been developed for determining fumonisins in corn and corn-based foods and feeds, including several techniques employing liquid chromatography, gas chromatography and thin layer chromatography, as well as enzyme-linked immunosorbent assay.\textsuperscript{8} However, the most frequently used technique is liquid chromatography (using different mobile phases) with fluorescence detection.\textsuperscript{3,4,6,8–20} As fumonisins do not fluoresce, their detection requires derivatization of the free amino group to form suitable fluorophors. Different reagents (fluorescamine,\textsuperscript{3} 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole,\textsuperscript{9} naphthalene-2,3-dicarboxaldehyde-KCN,\textsuperscript{4,9,10} 9-fluorenymethyl chloroformate,\textsuperscript{11} 6-aminoquinolyl N-hydroxysuccinimidylcarbamate\textsuperscript{12} and o-phthalaldehyde-2-mercaptoethanol (OPA–MCE),\textsuperscript{4,6,13–20} have been used for this purpose, but the last one seems to be the most appropriate. Chro-
matographic determination of fumonisins from corn and corn-based products is preceded by extraction from the sample, followed by a clean-up of the raw extract using different columns. Reversed-phase C18,4,16,18,20 and strong anion exchange6,9–14,19,20 solid-phase columns, as well as immunoaffinity columns (IMA)6,15,17,20 can be used for this purpose. Nowadays, IMA columns are widely used for clean-up. The main shortcomings of these columns are that they are for single-use only21 and a low recovery of about 75% in corn analysis.15 Fazekas et al.17 described a procedure for column regeneration. They used the regenerated column twice more, with a one-day regeneration period between the two uses. Thus, these authors used one column at least three times.

As can be seen from this short review, different analytical procedures for liquid chromatographic (LC) determination of fumonisins are described in the literature. For this reason, the objective of the present work was twofold: optimization of the experimental conditions for the liquid chromatographic determination of FB1 and FB2 and investigation of the possibility of multiple uses of immunoaffinity columns.

EXPERIMENTAL

Materials

All solvents used for the extraction of the fumonisins from corn samples, as well as for the preparation of the mobile phase were of HPLC grade. All chemicals used in the investigation were of reagent grade. Solutions were prepared in doubly deionized water, except when stated otherwise.

Fumonisins B1 (Sigma, from Fusarium moniliforme, approx. 98% TLC) and B2 (Sigma, from Fusarium moniliforme) were purchased as analytical standards. Calibrant solutions were prepared in acetonitrile–water (50:50, v/v) at a concentration of 100 μg/ml for both FB1 and FB2. The fumonisin calibrant solutions are stable for up to 6 months when stored at 4 °C.15 Stock solutions containing FB1 at 10 ng/μl and FB2 at 5 ng/μl were prepared by measuring 500 μl of calibrant solution FB1 and 250 μl of calibrant solution FB2 into 5 ml volumetric flasks and diluting to volume with acetonitrile–water (50:50, v/v). Working calibrant solutions were prepared by appropriate dilution of the stock solutions with acetonitrile–water (50:50, v/v). The standard solutions were stored at 4 °C.

Preparation of phosphate-buffered saline (PBS). 8.0 g NaCl, 1.2 g anhydrous Na2HPO4, 0.2 g KH2PO4 and 0.2 g KCl were dissolved in about 990 ml water, the pH was adjusted to 7.0 and the solution was diluted to 1 l.

Preparation of OPA–MCE reagent. 40 mg OPA (Sigma, min. 99%) were dissolved in 1 ml methanol, diluted with 5 ml 0.1 mol/l Na2B4O7, before 50 μl MCE (Serva) were added. This reagent is stable in the dark for up to 8 days in a capped, aluminum foil-covered vial.

LC mobile phase. Methanol–0.1 mol/l NaH2PO4 (Merck, extra pure) (78:22, v/v), with pH adjustment to 3.3 with o-phosphoric acid. The mobile phase was filtered through a 0.45 μm membrane (ISO-DISC™ Filters PTFE 25-4, Supelco).

Apparatus

The equipment consisted of an LC system – BioRad 2800 with a Supelcosil™ LC-18-DB column (250 × 4.6 mm id, particle size 5 μm) and a Hewlett Packard 1046A fluorescence detector, response time 4 s, flash frequency 220 Hz. The LC pump delivered a constant flow of 1 ml/min. The excitation wavelength was 220 nm and the emission was measured at 440 nm.

FumoniTest™ immunoaffinity columns (Vicam, Watertown, MA, USA).

The following equipment was used to perform the analysis: blender a commercial Laboratory
Waring blender (Waring-Dynamics, Corporation of America, New Hartford Connecticut, USA), sample evaporator (Devarot, Elektromedicina, Ljubljana, Slovenia), centrifuge (Tehtnica Železniki, Slovenia), 1.0 \( \mu \)m microfiber filters (Vicam, Watertown, MA, USA), 100 \( \mu \)l pipettes (Hamilton 710N), filter paper (Macherey-Nagel, Type 751, Düren, Germany), minishaker (IKA Worus INC, Wilmington, USA), and single position pump stand (Vicam, Watertown, MA, USA), pH meter (Sentron 2001, Netherlands), ultrasonic bath (for mobile phase degassing – 10 min before work, Sonis 3, Iskra, Slovenia).

**Procedure**

**Principle.** The fumonisins were extracted from corn with an acetonitrile–methanol–water mixture. After filtration and dilution, the crude extract was cleaned up on an IMA column, and the fumonisins were eluted with methanol. The final sample extract was derivatized with \( \alpha \)-phthaldehyde and 2-mercaptoethanol and analyzed by reversed-phase liquid chromatography with fluorescence detection.

**Preparation of a spiked corn sample.** A 1000 g corn sample (blank material, fumonisins B\(_1\) and B\(_2\) at \(< 0.05 \mu g/g\) was prepared by grinding in a laboratory mill to pass through a 0.8 mm sieve (> 93 \%) and subsequently well mixed. Then the corn sample was spiked with a known volume of stock standard solution of fumonisins, kept at room temperature for 60 min and then analyzed.

**Extraction and clean-up.** 20.0 g samples were extracted twice with 50 ml of acetonitrile–methanol–water (25:25:50, v/v/v) in a laboratory blender for two minutes. The combined extracts were centrifuged at 3000 \( \times \) g for 10 min, with subsequent supernatant filtration through a filter paper, after which 10 ml of the filtered extract was diluted with 40 ml of PBS. The diluted extract was then filtered through a 1.0 \( \mu \)m glass microfiber filter. 10 ml of the filtrate were then applied to an IMA column. After rinsing of the column with 10 ml of PBS, the fumonisins were eluted using 3 ml of methanol, at a rate of 1 drop per 10–15 seconds. The eluate was evaporated just to dryness at 60 °C. The purified residue was redissolved in 200 \( \mu \)l acetonitrile–water (50:50, v/v).

**Derivatization and liquid chromatography.** A 50 \( \mu \)l aliquot of the extract was mixed with 50 \( \mu \)l of the OPA–MCE reagent at room temperature and allowed to react with the reaction time one minute under stirring. 20 \( \mu \)l of the derivatized solution were injected into the LC system.

**Quantitative determination.** Calibration curves used for quantitative determination were constructed on the basis of the area under the FB\(_1\) and FB\(_2\) chromatographic peaks, using five FB\(_1\) and FB\(_2\) working standard solutions.

**Regeneration of the IMA columns.** One IMA column was used five times in a row. Each time, after the elution of toxins, the diluted extract was applied to the same column. Subsequently, the column was washed with 10 ml of PBS solution, leaving a part of the solution on the column. The column was regenerated at 4 °C for 1 day. The same procedure was used for the standard solution as well as for the spiked corn.

**Investigation of column efficiency by the use of the standard solution.** 30 \( \mu \)l stock standard solution were added to 10 ml PBS and the same clean-up procedure on IMA columns was applied as in the case of the spiked corn sample.

**RESULTS AND DISCUSSION**

**Preliminary study**

As has already been pointed out, the use of liquid chromatography with IMA column clean-up has been described in literature for the determination of fumonisins under different experimental conditions. For this reason, the preliminary research included the determination of the optimal conditions for the separation, detection and determination of fluorescent derivatives of FB\(_1\) and FB\(_2\). Since the use of IMA columns for corn extract clean-up enables the acquisition of chromato-
grams without matrix peaks, the optimization was performed with a standard solution containing a mixture of both fumonisins.

Firstly, the optimal mobile phase was investigated. Since it was found in the literature that the separation is the most efficient with the following mobile phase ratios: methanol–water–acetic acid (75:24:1, v/v/v), acetonitrile–water–acetic acid (50:50:1, v/v/v) and methanol–0.1 mol/l NaH₂PO₄ (77:23, v/v, pH 3.3 with o-phosphoric acid) these mobile phases were compared in this work. Although all three of them can be used for the determination of fumonisins, it appeared that the last mobile phase is the most suitable because it results in the most prominent separation of OPA and FB₁ peaks and the noise was lower, even though the use of NaH₂PO₄ causes certain technical problems. Since the literature data differ greatly in respect to the composition of the mobile phase varying from 80:20 (v/v), 77:23 (v/v), 75:25 (v/v) to 68:32 (v/v), which is probably due to the characteristics of the LC column, the optimal mobile phase composition was investigated under our experimental conditions (Fig. 2). As can be seen, decreasing the volume fraction of 0.1 mol/l NaH₂PO₄ in the mobile phase accelerates the analysis and enhances the efficiency of the elution of FB₂, on the one hand, but influences the separation efficiency of FB₁ from OPA on the other hand. Further improvement of the separation is probably possible with gradient elution, and this will be the subject of further research. As the mobile phase methanol–0.1 mol/l NaH₂PO₄ (78:22, v/v, pH 3.3 with o-phosphoric acid) was found to be the most appropriate (Fig. 2D), all other investigations were done using this mobile phase. The chromatograms shown in Fig. 3 support this choice of mobile phase composition, since under these conditions, the separation of the matrix peaks from FB₁ peaks is good. It should also be kept in mind that as even a small change in the mobile phase composition significantly influences the retention time, as well as the separation of OPA from FB₁, great attention should be devoted to its preparation.

Bearing in mind the progressive decay in the fluorescence intensity of OPA–fumonisin derivatives, particular attention was devoted to the reproducibility of the time between the addition of the OPA reagent and the injection into the LC system. However, different values for the derivatization time of fumonisins with the OPA reagent were noted in the literature (≤ 1, 1–2, 3, 5, 10 minutes), which are probably due to different concentration ratios of OPA and fumonisins. For this reason, the optimal time of derivatization with OPA–MCE was also investigated. It was established that varying the derivatization time between 1 to 10 min (all other applied conditions being the same) had no significant effect on the investigation results. This is very important, because small deviations in the derivatization time do not influence the repeatability of the determination.

By scanning of the content of the flow-through detector cell in which the peak had previously been “captured” by stopping the flow, the optimal excitation and emission wavelengths were determined. Although most authors use 335 nm as the excitation wavelength for the derivatized fumonisin molecule, the present results
indicate the necessity for excitation at 220 nm. Fig. 4 shows that the fluorescence peak areas in this case are considerably higher than when excitation was at 335 nm. As a result, the sensitivity of the determination is increased.

The influence of flow rate on the determination results was also examined (1.00 – 1.35 ml/min). It has been found that a flow rate of 1 ml/min separates the derivatized fumonisins and OPA in a sufficiently short time, so the use of higher flow rates, which cause a greater pressure increase on the column, is not necessary.

The effect of column temperature on the retention time and separation of fumonisins B1 and B2 was investigated with the aim of shortening the analysis time. Increasing the column temperature from ambient to 37 °C did not affect the separation efficiency, but it did shorten the analysis time by about ten minutes. Since some authors have indicated the possibility of decomposition of OPA–fumonisin derivatives at higher temperatures,14 separation at room temperature was chosen.

The linearity of the method was assessed by standards ranging from 0.125–2.00
ng/μl for FB₁ and 0.0625–1.000 ng/μl for FB₂ (derivatized solutions). The correlation coefficient and residual standard deviation values for the linear curves of both fumonisins are given in Table I.

**TABLE I.** Linearity curves, correlation coefficients and residual standard deviation values for the LC determination of FB₁ and FB₂

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Concentration range (ng/μl)</th>
<th>Linearity curve</th>
<th>Correlation coefficient</th>
<th>Residual standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB₁</td>
<td>0.125–2.00</td>
<td>( y = 11.64c - 0.57 )</td>
<td>0.999</td>
<td>0.37</td>
</tr>
<tr>
<td>FB₂</td>
<td>0.0625–1.000</td>
<td>( y = 8.30c - 0.29 )</td>
<td>0.999</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The linear curves are given according to the equation: \( y = mx + b \) (\( m \) – slope, \( b \) – intercept with the \( y \)-axis). The linearity for FB₁ and FB₂ determined by a five-point calibration.

The within-assay precision was evaluated by six repeated, separate measurements of a standard solution of FB₁ and of FB₂ (0.500 ng/μl FB₁ and 0.250 ng/μl FB₂). The relative standard deviation of the peak areas for FB₁ and FB₂ were 5.8 and 4.8 %, respectively. It is noteworthy that the relative standard deviation of the peak area for FB₂ are lower, although its area is about 3 times smaller and, hence, exactly the opposite would be expected.

The accuracy of the determinations is expressed as percentage recovery of known added amounts of FB₁ and FB₂ to corn. As the procedure for the determination of fumonisins in corn is relatively complex, the percentage recovery for the IMA column and especially for the entire procedure, including fumonisins extraction from spiked corn was investigated. The procedure for investigating the column efficiency using standard solution is described in the Experimental part. The recoveries and relative standard deviations for both fumonisins are given in Table...
II. As can be seen, if the elution was performed with only 1.5 ml of methanol (as declared by the manufacturer) recoveries of 70.3 % for FB1 and 75.2 % for FB2 were obtained, which are in agreement with the results of other authors. However, if elution is performed with a further 1.5 ml of methanol, the total recovery is about 30 % higher and values of 100.8 % for FB1 and 105.6 % for FB2 were obtained, which leads to the conclusion that elution with 3 ml of methanol is more efficient, and, thus, this amount was used in further work. It was also found that the elution flow rate for fumonisins must be slower than that recommended by the producer, i.e., 1 drop per 10–15 seconds.

**TABLE II.** The effect of eluent volume from an IMA column on the recovery of 300 ng FB1 (equivalent to 0.75 µg/g FB1 in corn) and 150 ng FB2 (equivalent to 0.375 µg/g FB2 in corn)

<table>
<thead>
<tr>
<th>Determination number</th>
<th>Recoverya (%)</th>
<th>Recoveryb (%)</th>
<th>Recoveryc (%)</th>
<th>Recoverya (%)</th>
<th>Recoveryb (%)</th>
<th>Recoveryc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.7</td>
<td>39.0</td>
<td>92.7</td>
<td>62.0</td>
<td>42.7</td>
<td>104.7</td>
</tr>
<tr>
<td>2</td>
<td>78.0</td>
<td>9.3</td>
<td>87.3</td>
<td>85.3</td>
<td>10.7</td>
<td>96.0</td>
</tr>
<tr>
<td>3</td>
<td>85.7</td>
<td>33.7</td>
<td>119.4</td>
<td>96.0</td>
<td>30.0</td>
<td>126.0</td>
</tr>
<tr>
<td>4</td>
<td>69.3</td>
<td>41.7</td>
<td>111.0</td>
<td>68.0</td>
<td>38.7</td>
<td>106.7</td>
</tr>
<tr>
<td>5</td>
<td>64.7</td>
<td>28.7</td>
<td>93.4</td>
<td>64.7</td>
<td>30.0</td>
<td>94.7</td>
</tr>
<tr>
<td>Mean</td>
<td>70.3</td>
<td>30.5</td>
<td>100.8</td>
<td>75.2</td>
<td>30.4</td>
<td>105.6</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>17.5</td>
<td>22.2</td>
<td>13.6</td>
<td>19.6</td>
<td>40.5</td>
<td>11.9</td>
</tr>
</tbody>
</table>

*Eluted with 1.5 ml methanol; \(^b\)eluted with a further 1.5 ml methanol; \(^c\)total elution with 3.0 ml methanol

The detection limit measured as signal-to-noise ratio (3:1) was 0.025 ng/µl for FB1 and 0.065 ng/µl for FB2, which corresponds, respectively, to 0.025 and 0.065
μg/g fumonisins in corn, which is significantly lower than the recommended tolerance value.\textsuperscript{5}

As the fumonisins content may, in some cases, be very low in a sample, the effect of injecting different volumes of the same working solution (0.125 ng/μl FB\textsubscript{1} and 0.0625 ng/μl FB\textsubscript{2}) on the results of the determination was investigated. A linear dependency was found in the volume range from 20 to 80 μl (the correlation coefficient for FB\textsubscript{1} is 0.999 and for FB\textsubscript{2} 0.997).

Results of tests with the regenerated columns

Furthermore, because IMA columns are relatively expensive (they are according to the producer for single-use only),\textsuperscript{21} it is desirable to reuse them as many times as possible. For this reason, great attention was devoted in this study to the examination of the possibility of multiple column regeneration.

The investigation of the possibility of IMA column reuse for standard FB\textsubscript{1} and FB\textsubscript{2} solutions applied in PBS solvent showed that IMA columns can be used at least five times in a row. This is very significant because it is possible to use the same column for successive analyses without having to wait one day for regeneration, as was suggested by Fazekas \textit{et al.}\textsuperscript{17} It was also found that it is possible to use the column twice more after regeneration with phosphate buffered saline for 1 day at 4 °C. Although the reproducibility of determination is somewhat lower after regeneration, according to one-way analysis of the variance, there are no significant differences (\( p = 0.05 \)) in respect to the accuracy between a new column and the same column after the first and second regeneration.

TABLE III. Recovery of 1 μg/g FB\textsubscript{1} and 0.5 μg/g FB\textsubscript{2} applied as solutions for spiked corn, in a row and after regeneration of the IMA column

<table>
<thead>
<tr>
<th>Determination number</th>
<th>New column</th>
<th>1. Regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FB\textsubscript{1}/%\textsuperscript{a}</td>
<td>FB\textsubscript{2}/%\textsuperscript{b}</td>
</tr>
<tr>
<td>1</td>
<td>99.4</td>
<td>87.2</td>
</tr>
<tr>
<td>2</td>
<td>91.2</td>
<td>89.6</td>
</tr>
<tr>
<td>3</td>
<td>88.9</td>
<td>89.7</td>
</tr>
<tr>
<td>4</td>
<td>84.5</td>
<td>87.0</td>
</tr>
<tr>
<td>5</td>
<td>80.9</td>
<td>89.1</td>
</tr>
<tr>
<td>Mean</td>
<td>89.0</td>
<td>88.5</td>
</tr>
<tr>
<td>RSD/%</td>
<td>7.9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 400 ng FB\textsubscript{1} standard applied to the column; \textsuperscript{b} 200 ng FB\textsubscript{2} standard applied to the column

For the investigation of the reusability of an IMA column for spiked corn (Fig. 3B), a corn sample was used in which the FB\textsubscript{1} content was beneath the detection
limit, while FB2 was not detected (Fig. 3A). It was found in this case as well that it is possible to perform five clean-ups of the corn extract in a row, without column regeneration and a further five after the first regeneration (Table III). However, column clogging caused by the corn matrix occurs after 10 clean-ups. Hence, in the case of the corn samples, it is possible to perform 10 clean-ups of corn extracts with one IMA column. As can be seen, the mean recoveries of FB1 and FB2 from corn spiked with FB1 at 1.0 µg/g and with FB2 at 0.5 µg/g (on the basis of 10 measurements) were 88.7% (RSD 10.2%) and 90.5% (RSD 6.1%), respectively.

<table>
<thead>
<tr>
<th>Content/µg g⁻¹</th>
<th>FB1</th>
<th>FB2</th>
<th>FB1</th>
<th>FB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.25</td>
<td>92.5</td>
<td>113.4</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>0.50</td>
<td>89.0</td>
<td>88.5</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>1.00</td>
<td>81.9</td>
<td>72.0</td>
<td></td>
</tr>
</tbody>
</table>

The influence of the content of fumonisins on the recovery was also investigated (Table IV). As can be seen, the recovery decreases with increasing content, but is still significantly higher than the value found in the literature.¹⁵

CONCLUSION

The IMA column coupled with liquid chromatography method was optimized for the determination of fumonisins. It was found that the most suitable mobile phase for their separation was methanol–0.1 mol/l NaH₂PO₄ (78:22, v/v, pH 3.3 with o-phosphoric acid) at a flow rate of 1 ml/min. The time of derivatization (1–10 min) does not have a significant effect on the results. The optimal excitation wavelength is 220 nm. Elution of the IMA columns with 3 ml of methanol is more efficient, and the elution flow rate for fumonisins must be 1 drop per 10–15 seconds.

Immunoaffinity columns, designated for single-use only, can be used at least five times without regeneration and a further five times after regeneration with phosphate buffered saline for 1 day at 4 °C for corn analysis.

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IZVOD
ODREŽIVAWE FUMONIZINA B1 I B2 U UZORCIMA KUKURUZA TEČNOM 
HRATOMOGRAFIJOM UZ VI[ESTRUKU PRIMENU IMUNOAFINITETNIH 
KOLONA ZA PRE^I[AVAWE 

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Фумонизини су група микотоксини које производују углавном плесни Fusarium moniliforme и најчешће се срећу у кукурузу и производима од кукуруза. Изазивају више различитих болести код животиња, због чега је веома важно располагати са тачним и поузданим методама за њихово одређивање. У литератури је описан већи број различитих поступака за одређивање садржаја фумонизина B1 (FB1) и B2 (FB2) течном хроматографијом. Из тог разлога циљ овога рада је био одређивање FB1 i FB2 у узorcima кукуруза течном хроматографијом уз вишеструку примену имунонаправнотивних (IMA) колона за пречишћавање сировог екстракта. Након оптимизације хроматографског одређивања дериватизованих фумонизина са o-фталалдегидом и 2-меркаптоетанолом, испитана је ефикасност вишеструког применених IMA колона (са и без регенерације) за пречишћавање сировог екстракта обогаћеног кукуруза. Нађено је да се IMA колове, које су декларисане за једнократну употребу, могу успешно користити најмање пет пута без регенерације и још пет пута након регенерације. Регенерација се састојала у дршавању раствора фосфатног пуфера у IMA колови један дан на 4 °C. Ефикасност регенерације је тестирана на основу тачности и прецизности резултата одређивања FB1 и FB2. Нађено је да је ефикасност одређивања фумонизина (средња вредност десет мерених) из кукуруза обогаћеног са 1,0 μg/g FB1 и 0,5 μg/g FB2 88,7 % (RSD 10,2 %), односно 90,5 % (RSD 6,1 %).

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REFERENCES
9. P. M. Scott, G. A. Lawrence, J. AOAC Int. 77 (1994) 541