Liquid chromatographic determination of fumonisins B1 and B2 in corn samples after reusable immunoaffinity column clean-up

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Abstract. The possibility of the liquid chromatographic determination of fumonisins B1 (PB1) and B2 (FB2) 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 FB1 and FB2 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 FB1 and FB2 as well as the reproducibility of the determinations. The mean recoveries of FB1 and FB2 from corn spiked with FB1 at 1.0 ng/g and with FB2 at 0.5 pg/g (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 fumonism 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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cancer in people from South Africa and China. The International Agency of Research of Cancer has classified *Fusarium moniliforme* toxins as potential carcinogens for humans (class 2B carcinogens), similar to ochratoxin A. An official tolerance value for dry corn products (1 ug/g) has been issued only in Switzerland, while only recommendations have been issued in the United States and France. 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](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). 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. However, the most frequently used technique is liquid chromatography (using different mobile phases) with fluorescence detection. As fumonisins do not fluoresce, their detection requires derivatization of the free amino group to form suitable fluorophors. Different reagents (fluorescamine, 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole, naphthalene-2,3-dicarboxaldehyde-KCN, 9-fluorenylmethyl chloroformate, 6-aminoquinolyl N-hydroxysuccinimidyldiarylcarbamate and ophthalaldehyde-2-mercaptoethanol (OPA-MCE), 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, C16, C18, and strong anion exchange solid-phase columns, as well as immunoaffinity columns (IMA) 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 only and a low recovery of about 75% in corn analysis. Fazekas et al. 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 FE₂ 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. Stock solutions containing FB₁ at 10 ng/ul and FB₂ at 5 ng/ul 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 OPAMCE reagent. 40 mg OPA (Sigma, min. 99%) were dissolved in 1 ml methanol. diluted with 5 ml 0.1 mol/1 Na₂B₂O₄, 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/1 NaH₂PO₄ (Merck, extra pure) (78:22, v/v), with pH adjustment to 3.3 with orthophosphoric acid. The mobile phase was filtered through a 0.45 (am 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 x 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 (Devorot, Elektromedica, Ljubljana, Slovenia), centrifuge (Tehtnica Zelezni, Slovenia), 1.0 pm microfiber filters (Vicam, Watertown, MA, USA), 100 ul 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 o-phthalaldehyde 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 B1 and B2 at < 0.05 fig/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 x 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 jim glass microfiber filter. 10ml 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 ul 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 ul 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 FB1 (and FB2) chromatographic peaks, using five FB1 and FB2 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 1 Om 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 FB1 and FB2. 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/1 NaH$_2$PO$_4$ (77:23, v/v, pH 3.3 with o-phosphoric acid) these mobile phase 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 FB$_1$ peaks and the noise was lower, even though the use of NaH$_2$PO$_4$ 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), 75:25 (v/v), and 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/1 NaH$_2$PO$_4$ in the mobile phase accelerates the analysis and enhances the efficiency of the elution of FB$_2$, on the one hand, but influences the separation efficiency of FB$_1$ 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/1 NaH$_2$PO$_4$ (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 FB$_1$ 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 FB$_1$, 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, 14, 17, 20 and 16 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
Fig. 2. Effect of different mobile phase ratios MeOH-0.1 mol/1 NaH$_2$PO$_4$ on the separation of 20 ng FB, and 10 ng FB, (v/v): (A) 90:10; (B) 85:15; (C) 80:20; (D) 78:22; (E) 77:23. (pH 3.3, flow rate 1 ml/min, excitation wavelength 335 nm, emission wavelength 440 nm, derivatization 1 min).

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 62 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, separation at room temperature was chosen.

The linearity of the method was assessed by standards ranging from 0.125-2.00
ng/ul for FBj and 0.0625-1.000 ng/ul 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 FBj and FB2.

<table>
<thead>
<tr>
<th>Mycothxin (ng/ul)</th>
<th>Concentration range</th>
<th>Linear curve</th>
<th>Correlation coefficient</th>
<th>Residual standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBj</td>
<td>0.125-2.00</td>
<td>$y = 0.64c - 0.57$</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 FBj and FB2 determined by a five-point calibration.

The within-assay precision was evaluated by six repeated, separate measurements of a standard solution of FBj and of FB2 (0.500 ng/ul FBj and 0.250 ng/ul FB2). The relative standard deviation of the peak areas for FBj 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 FBj 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.
11. As can be seen, the elution was performed with only 1.0 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>
<thead>
<tr>
<th>Determination number</th>
<th>Recovery (%)</th>
<th>Recovery (%)</th>
<th>Recovery (%)</th>
<th>Recovery (%)</th>
<th>Recovery (%)</th>
<th>Recovery (%)</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>
</tbody>
</table>

The detection limit measured as signal-to-noise ratio (3:1) was 0.025 ng/jil for and 0.065 ng/jil for FB2, which corresponds, respectively, to 0.025 and 0.065
(ag/g fumonisins in corn, which is significantly lower than the recommended tolerance value. 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/jil FB\textsubscript{1} and 0.0625 ng/jil FB\textsubscript{2}) on the results of the determination was investigated. A linear dependency was found in the volume range from 20 to 80 jìl (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), 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 et al. 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>
<thead>
<tr>
<th>Determination number</th>
<th>New column</th>
<th>1 . Regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FB\textsubscript{1}%</td>
<td>FB\textsubscript{2}%</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>
</tbody>
</table>

RSD/%

7.9 1.5 13.1 8.2

\*400 ng FB\textsubscript{1} standard applied to the column; \*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 FBi and FE$2$ from corn spiked with FBi at 1.0 jag/g and with FB2 at 0.5 (jg/g (on the basis of 10 measurements) were 88.7 % (RSD 10.2 %) and 90.5 % (RSD 6.1 %), respectively.

TABLE IV. Recovery of FB, and FB$_2$ applied as solutions for spiked corn

<table>
<thead>
<tr>
<th>Content/jg g</th>
<th>FB$_1$</th>
<th>FB$_2$</th>
<th>FB$_1$</th>
<th>FB$_2$</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 average of two measurements

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 NaH$_2$O$_4$ (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.

Immunobaffinity columns, designated for single-use only,$^{21}$ 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

ODREĐIVANJE FUMONIZINA B, I B2 U UZORCIMA KUKURUZA TECNOM HROMATOGRAFIJOM UZ VIŠEST RUKU PRIMENU IMUNOAFINITETNIH KOLONA ZA PRECIŠCAVANJE

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Fumonizini su grupa mikotoksina koje produkuju uglavnom plesni Fusarium moniliforme i najčešće se sreću u kukuruzu i proizvodima od kukuruza. Izazivaju više različitih bolesti kod životinja, zbog čega je veoma važno raspolagati sa tacnim i pouzdanim metodama za njihovo određivanje. U literaturi je opisan veći broj različitih postupaka za određivanje sadržaja fumonizina B1 (FB1) i B2 (FB2) tecnom hromatografijom. Iz tog razloga cilj ovoga rada je bio određivanje FB1 i FB2 u uzorcima kukuruza tecnom hromatografijom uz višestruku primenu imunoafinitetnih (IMA) kolona za precišćavanje sirovog ekstrakta. Nakon optimizacije hromatografskog određivanja derivatizovanih fumonizina sa o-taldialdehidom i 2-merkaptoetanolom, ispitana je efikasnost višesugasto primenjenih IMA kolona (sa i bez regeneracije) za precišćavanje sirovog ekstrakta obogacenog kukuruza. Nadeno je da se IMA kolone, koje su deklarirane za jednokratnu upotrebu, mogu uspešno koristiti najmanje pet puta bez regeneracije i još pet puta nakon regeneracije. Regeneracija se sastojala u držanju rastvora fosfatnog pufera u IMA koloni jedan dan na 4 °C. Efi̇kasanost regeneracije je testirana na osnovu tacnosti i preciznosti rezultata određivanja FB1 i FB2. Nadeno je da je efikasnost određivanja fumonizina (srednja vrednost deset merenja) iz kukuruza obogacenog sa 1,0 ng/g FB1 i 0,5 ng/g FB2 88,7 posto (RSD 10,2 posto), odnosno 90,5 posto (RSD 6,1 posto).


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