Determination of Aflatoxins in Rice Samples by Ultrasound-Assisted Matrix Solid-Phase Dispersion

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This work describes the application of ultrasound-assisted matrix solid-phase dispersion as an extraction and sample preparation approach for aflatoxins (B1, B2, G1 and G2) and subsequent determination of them by high-performance liquid chromatography–fluorescence detection. A Box–Behnken design in combination with response surface methodology was used to determine the affecting parameters on the extraction procedure. The influence of different variables including type of dispersing phase, sample-to-dispersing phase ratio, type and quantity of clean-up phase, ultrasonication time, ultrasonication temperature, nature and volume of the elution solvent was investigated in the optimization study. C18, primary–secondary amine (PSA) and acetonitrile were selected as dispersing phase, clean-up phase and elution solvent, respectively. The obtained optimized values were sample-to-dispersing phase ratio of 1 : 1, 60 mg of PSA, 11 min ultrasonication time, 30°C ultrasonication temperature and 4 mL acetonitrile. Under the optimal conditions, the limits of detection were ranged from 0.09 to 0.14 ng g⁻¹ and the precisions [relative standard deviation (RSD%)] were <8.6%. The recoveries of the matrix solid-phase dispersion process ranged from 78 to 83% with RSD <10% in all cases. Finally, this method was successfully applied to the extraction of trace amounts of aflatoxins in rice samples.

Introduction

Mycotoxins are a large and varied group of mold-secondary metabolites. Several hundred different mycotoxins have been discovered so far, exhibiting great structural diversity, which result in different chemical and physicochemical properties. Aflatoxins, a variety of mycotoxins, have remained at the center of attention due to their frequent occurrence and severe effects on animal and human health (1–3). IACs are usually preferred to SPE due to their better performances in terms of yield and quantification limit. However, these methods are relatively expensive and time consuming. Large amounts of solvent consumption, a high expertise requirement level and the use of expensive disposable cartridges are other notable drawbacks of applying IACs (11, 13–16). Rubert et al. (17) reported matrix solid-phase dispersion (MSPD) for the extraction of aflatoxins in cereals. This sample preparation method, alternative to the more classical methods, has reduced sample size and solvent consumption and also can be used to carry a multi mycotoxin extraction (17, 18). Also, an enzyme-linked immunosorbent assay method cannot exactly corroborate the existing of the toxins; neither definitely quantify them (19).

Even very small quantities of aforementioned toxins induce irrepairable effects so there is a substantial need to apply a method for determination of trace and ultratrace amounts of toxins in natural samples. Therefore, a simple, rapid and sensitive method such as high-performance liquid chromatography with fluorescence detection (HPLC-FLD) combined with pre- or postcolumn derivatization is currently one of the most extensively favored methods for the determination of aflatoxins due to its great versatility in the analysis of complicated matrices (20–22). Several methods of derivatization are reported in the literatures, including precolumn derivatization with trifluoroacetic acid (23) and postcolumn treatment with iodine (24). These methods offer a consumption by human. The maximum limits established by Iran regulations for AFB1 and total aflatoxins in rice are 5 and 30 ng g⁻¹, respectively (9).

To satisfy regulatory limits, a great number of analytical approaches have been developed for the identification and determination of implied mycotoxins in diverse complex samples, such as food, feed and biological matrices. Besides the complication of sample matrices, owing to the trace levels of aflatoxins in cereals; an appropriate sample preparation procedure has seemed to be necessary for the elimination of the sample matrix and preconcentration of the analytes prior to instrumental analysis. Until now a vast number of procedures have been developed for the extraction, preconcentration and purification of aflatoxins from sample matrices.

Extraction of aflatoxins can be performed by using a mixture of water and organic solvent, e.g., methanol, acetonitrile, and so on (10–12). Several methods have been applied for exclusion of matrix interferences. Nowadays, solid-phase extraction (SPE) or immunoaffinity columns (IACs) that are commercially available, help to simplify protocols, improve selectivity and thus performance characteristics (3). IACs are usually preferred to SPE due to their better performances in terms of yield and quantification limit. However, these methods are relatively expensive and time consuming. Large amounts of solvent consumption, a high expertise requirement level and the use of expensive disposable cartridges are other notable drawbacks of applying IACs (11, 13–16).

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number of drawbacks, such as utilization of toxic reagents, instability of the derivatives and a low day-to-day reproducibility (25). In order to overcome these disadvantages, an online photochemical derivatization system has been introduced (26). It has been reported that online photochemical derivatization coupled to fluorescence detection enhanced efficiency and performance of HPLC (25–27). Derivatization of aflatoxins (AFTs) by using an electrochemical cell generates derivatizing agent (bromine) in the mobile phase. It causes rapid derivatization at ambient temperature. This procedure has several advantages. It does not need heating block, daily preparation of derivatizing reagent, using additional pump to add derivatizing reagent and handling with halogenated solutions (e.g., iodine). The stability problems of mobile phase were not observed even after addition of potassium bromide and nitric acid. Low corrosion of pumps, low maintenance costs, high increment of AFT fluorescence activity, fast derivatization (4 s) at ambient temperature and high reproducibility of results are also the other advantages of derivatization with electrochemical cell (28, 29). Ultrasonic radiation is a powerful tool for the acceleration of the mass transfer process of the analytes between the solid sample and the extraction solvent, resulting in an increase in the extraction efficiency in a short time (30–32).

The aim of this work was to establish a reliable, simple, time saving and cost-effective extraction procedure based on MSPD assisted by ultrasonic radiation for aflatoxins in rice samples. The MSPD method for extraction of aflatoxins was developed by optimizing dispersing phase, clean-up phase, elution solvent and its volume, time and temperature of ultrasonication. Moreover, finding a method that does not suffer from matrix interfering effect so much and obtaining high recoveries for the four mycotoxins were the aim as well. Determination of the analytes was performed by using HPLC-FLD and photochemical post-column derivatization. Consequently, the results illustrated that the new method is an efficient extraction procedure for aflatoxins determination in rice samples. Also the present method was compared with other methods, which are mentioned in the Introduction section. The comparison results showed that the proposed method is more sensitive [lower limits of detection (LODs)] in some cases compared with the other reported methods. Also, it has been shown that the relative standard deviations (RSDs) of the present method are similar to those of other methods or are better. Hence, the analytical performance of this method is acceptable.

Experimental

Chemical and reagents

The standards of AFB1, AFB2, AFG1 and AFG2 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock standard solution of multitoxin (1,000 ng mL$^{-1}$) was prepared in acetonitrile and stored at refrigerator. The mixed working solutions of aflatoxins were prepared daily by diluting the standard solutions prior to use. Acetonitrile (HPLC grade), methanol (HPLC grade), n-hexane, ethyl acetate, dichloromethane and acetone were supplied by Merck (Darmstadt, Germany). Silica, alumina, phenyl, octyl-silica (C$_{8}$, 50 µm) and octadecyl-silica (C$_{18}$, 50 µm) were supplied by Analisis Vinicos (Tomelloso, Spain) and used as solid phases for ultrasound-assisted matrix solid-phase dispersion (UA-MSPD). Florisil® (60–100 mesh) was obtained from Aldrich (Steinheim, Germany). Graphitized carbon black (GCB) was obtained from Supelco Inc. (Bellefonte, PA, USA). Deionized water was prepared using a Milli-Q system from Millipore (Bedford, MA, USA). Rice samples were purchased from a local market (Qazvin, Iran).

HPLC instrument

Separation and determination of aflatoxins levels were performed on the Wellchrom HPLC system from Knauer Company (Berlin, Germany). The HPLC instrument consisted of an online K-5020 degasser, a smart line pump1000, a 6-port/3-channel injection valve equipped with a 100-µL loop and an RF-10XAL fluorescence detector and an electrochemical cell for the postcolumn bromine derivatization of AFTs (Model KB Libios cell, Libios, France). EZ Chrom Elute 2000 was used to process chromatographic data. Chromatographic separations were carried out using a Capital HPLC column (Scotland, UK) ODS-H C18 (250 × 4.6 mm i.d., 5 µm) at room temperature. A mixture of methanol : acetonitrile : water (20 : 20 : 60, v/v/v), containing 119 mg of potassium bromide and 100 µL of 65% nitric acid, with a flow rate of 1.0 mL min$^{-1}$ was mobile phase in isocratic elution mode and filtered through a Millipore 0.22-µm membrane filter before use. Excitation and emission wavelengths were set at 362 and 440 nm, respectively.

UA-MSPD procedure

Samples (each 200 g) were prepared using a food processor and mixed thoroughly. For the preparation of spiked samples, 1.0 mL of the standard working solution was added to 1.0 g of sample. Then, they were allowed to stand at room temperature for 3 h for the evaporation of the solvent; therefore, the equilibration between the aflatoxins and the rice samples was achieved (17). Afterwards, portions of 1.0 g of spiked and non-spiked samples were weighed and placed into a glass mortar (20 mL) and were gently blended with 1 g of C$_{18}$ for 5 min using a pestle, until a mixture became homogeneous. Subsequently, the homogeneous mixture was introduced to the top of 60 mg packed primary—secondary amine (PSA) (as clean-up phase) located in a 10 mL glass syringe (100 × 9 mm i.d.) and slightly compressed with a syringe plunger. Thereafter, the elution solvent (4 mL acetonitrile) was loaded while both ends of the cartridge were closed with polypropylene caps and the homemade column was immersed in an ultrasonic bath at 30°C for 11 min. After sonicating for a predetermined time, the extract was collected in a conical glass tube by applying a slight vacuum and then the extract was evaporated using a gentle stream of nitrogen gas. Ultimately, the residue was dissolved in 0.5 mL of acetonitrile and filtered through a 0.45-µm filter before injection into the HPLC-FLD system.

Optimization strategy

There are a number of factors, such as type of dispersing phase, sample-to-dispersing phase ratio, type and quantity of clean-up phase, ultrasonication time, ultrasonication temperature, nature and volume of the elution solvent that affect the UA-MSPD process. Primarily, one variable at a time method was carried out to select a proper dispersing phase, clean-up phase and elution solvent. Subsequently, in order to investigate the existing
interaction among these variables, a Box–Behnken design (BBD) was employed to develop the corresponding response surface equation (33). The experimental design matrix and data analysis were carried out by Statgraphics Plus Package, version 5.1.

**Results**

In this study, UA-MSPD in combination with HPLC-FLD was performed for the extraction and quantification of aflatoxins in rice samples. This method was developed by optimizing dispersing phase, clean-up phase, elution solvent and its volume, time and temperature of ultrasonication.

**Selection of the dispersing phase**

The effect of various dispersing phases on aflatoxins recovery was investigated, including C<sub>18</sub>-silica (octadecyl-silica) and C<sub>8</sub>-silica (octyl-silica) as reversed-phase sorbents and silica gel, florisil, alumina and phenyl as normal-phase non-bonded sorbents. Figure 1A exhibits recoveries obtained by using different dispersing phases. As it can be seen when C<sub>18</sub> was used, the recovery percentages of target compounds were >75%; which is clearly high compared with the recoveries gained by using other dispersing phases. Hence, C<sub>18</sub> was the best chosen dispersing phase for UA-MSPD since it gave the best recovery averages for all the studied aflatoxins. Particularly, aflatoxins are non-polar compounds; thus non-polar phases such as C18 are the most suitable for the extraction of these compounds.

**Selection of clean-up phase**

Rice samples show very complicated matrixes; hence, the addition of a clean-up phase in the bottom part of the syringe is vital. The effect of two diverse clean-up phases on aflatoxins recovery, including GCB and PSA, was studied under the extraction condition of: 1.0 g of sample spiked with 2.0 ng g<sup>−1</sup> of each aflatoxin, 5 mL acetonitrile, sample to adsorbent ratio 1 : 1, ultrasonication time 10 min and ultrasonication temperature 25°C. The results revealed that sequential clean-up by PSA was more efficient than GCB in removing interferences from rice sample. The recovery values and RSD% were 60 ± 6.5, 72 ± 5.1, 58 ± 5.9 and 64 ± 7.0% for AFB2, AFG2, AFB1 and AFG1, respectively, in the case of GCB as a clean-up phase. These values were 81 ± 4.7, 84 ± 6.2, 78 ± 7.1 and 79 ± 5.0 for AFB2, AFG2, AFB1 and AFG1, respectively, in the case of PSA as a clean-up phase. PSA sorbent has polar functional groups and high adsorption capacity so it can remove matrix components such as fatty acids, sugars and starch effectively.

**Selection of elution solvent**

The nature of the elution solvents is an important parameter since the target analytes should be efficiently eluted while the remaining matrix components should be retained in the solid sorbent (17). In this study, several solvent with very different polarities such as acetonitrile, methanol, acetone, ethyl acetate, n-hexane and dichloromethane were tested as the elution solvent. Figure 1B illustrates recoveries obtained by different elution solvents. As it is shown by using acetonitrile, the recovery percentages of aflatoxins were >80%, which is dramatically higher than the recoveries of other elution solvents. Acetonitrile can desorb aflatoxins without coelution of polar and non-polar matrix components since it has medium polarity. Hence, acetonitrile was chosen as the best elution solvent since it gave the highest recoveries, cleanest extracts and chromatograms.

**Figure 1.** (A) Effect of different dispersing phases on recovery of aflatoxins. Condition: 1.0 g of sample spiked with 2.0 ng g<sup>−1</sup> of each aflatoxin, 5 mL acetonitrile, sample to adsorbent ratio 1 : 1, ultrasonication time 10 min and ultrasonication temperature 25°C. (B) Effect of different elution solvents on recovery of aflatoxins; all conditions are similar to (A) except elution solvent.

**The effect of sample pH**

Aflatoxins are non-ionizable compounds (according to their structure) due to their functional groups so they can be extracted under a wide range of pH (3–11) but at strong acidic (<3) and basic (>11) pHs aflatoxins would decompose (8).
Hence, the procedure was accomplished without pH adjustment of rice samples.

**Discussions**

**Multivariate optimization of UA-MSPD variables**

In the following step, a BBD was used to optimize the five remained variables (sample-to-dispersing phase ratio, quantity of clean-up phase, ultrasonication time, ultrasonication temperature and volume of the elution solvent). The preselected levels of the factors and experimental runs with their responses are given in Supplementary data, Tables SI and SII. BBD is a suitable approach for the exploration of quadratic response surfaces and construction of a second-order polynomial model (34), which can be described as follows:

\[
Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2
\]

where \(x_1, x_2 \) and \(x_3\) are the independent variables, \(\beta_0\) is an intercept, \(\beta_1 - \beta_{33}\) are the regression coefficients and \(Y\) is the response (recovery %). The number of experiments (\(N\)) is defined by the expression below:

\[
N = 2K(K - 1) + C_0
\]

where \(K\) is the number of variables, and \(C_0\) is the number of center points (35). In this study, \(K\) and \(C_0\) were set at 5 and 12, respectively; which meant that 52 experiments had to be performed. These experiments were carried out in two separate blocks. The upper and lower values attributed to each factor were chosen by preliminary tests empirically.

The results of the analysis of variance were appraised for determining the main effects (36). The normalized results of the experimental design were evaluated at the 95% level of confidence (\(P = 0.05\)) and were analyzed by standardized Pareto chart (Figure 2). The standard effect was estimated for computing the \(t\) statistic for each effect. The vertical line on the plot determines statistically significant effects (37). The bar extracting beyond this line corresponds to the effects that are statistically significant at 95% level of confidence (\(P = 0.05\)) (38–40).

Furthermore, the positive or negative sign (corresponding to a colored or colorless response) can be enhanced or reduced, respectively, when passing from the lowest to the highest level set for the specific factor. According to Figure 2, the volume of elution solvent was the most significant factor having a positive effect on the extraction efficiency in this study. Also, as it is shown in Figure 2, ultrasonication time is the next significant factor, which has a positive effect on the extraction efficiency of aflatoxins.

The response surface methodology approach was used to analyze simultaneous effects of different variables on the extraction efficiency and extraction recoveries of aflatoxins. Some of the response surface plots are depicted at Figure 3 and Supplementary data, Figure S1, and their curvatures indicate the interaction between the factors. The plot of eluent volume vs. ultrasonication time is shown in Figure 3. It indicates that longer ultrasonication time and higher solvent volumes are optimal for the UA-MSPD process. The recoveries of analytes increased rapidly to nearly 87% by increasing the eluent volume to 4 mL, and then reached to an equilibrium value with increasing the elution volume further. As it’s obvious recovery increased by increasing the ultrasonication time to 11 min, after which the recovery decreased due to the possible degradation of aflatoxins and evaporation of acetonitrile. Supplementary data, Figure S1A show the plot of sample to dispersing phase ratio vs. ultrasonication temperature. According to this, plot recoveries were increased up to 1 : 1 ratio and then were decreased at higher ratio (1 : 2), probably due to
the high dispersion of the sample into the solid-phase dispersant (17, 32). In the ratio of 2:1, matrix interference decreased the extraction recovery of each aflatoxin. As expected, the experimental results showed that the extraction efficiency of aflatoxins increased with increasing ultrasonication temperature up to 30°C. This can be explained by the fact that an increase in ultrasonication temperature would increase the diffusion coefficient of aflatoxins so that the equilibrium would be achieved faster at higher temperatures. At temperature >30°C, the recovery decreased due to the possible degradation of aflatoxins and evaporation of organic solvent. Supplementary data, Figure S1B reveal the plot of ultrasonication temperature vs. PSA (clean-up phase) amount. It expresses that increasing PSA amount up to 60 mg was more favorable for extraction of aflatoxins, due to achieving effective clean-up. At higher amounts, PSA posed good ability for eliminating interference and gave cleaner extracts, but the recoveries of aflatoxins were unsatisfactorily low. According to the overall results of the optimization study, the following experimental conditions were determined: eluent volume, 4 mL; ultrasonication time, 11 min; ultrasonication temperature, 30°C; sample-to-dispersing phase ratio, 1:1 and amount of clean-up phase, 60 mg PSA.

**Validation of the method**

The analytical features of the UA-MSPD method were estimated by determining analytical figures of merit such as calibration curve, LODs, limits of quantification (LOQs), precision and recovery under the optimum conditions as listed in Table I. LODs and LOQs were determined through equation 3

\[ \text{LOD} = \frac{3S_b}{m} \]

\[ \text{LOQ} = \frac{10S_b}{m} \]

where \( S_b \) is the standard deviation of the blank signal and \( m \) is the slope of calibration curve after extraction (33), which was 0.04, 0.05, 0.11 and 0.14 ng g\(^{-1}\) for AFB2, AFG2, AFB1 and AFG1, respectively. LOQ is the lowest concentration that meets LOD criteria with a signal-to-noise ratio of 10 and was in the range of 0.12–0.56 ng g\(^{-1}\) for different analytes (41). As it can be seen that LODs and LOQs were lower than the established maximum residue limits or at least similar to them, indicating that the proposed method is adequate for determination of aflatoxins in the rice samples. Linear dynamic ranges (LDRs) were obtained within the range of 0.2–5 ng g\(^{-1}\). Correlation of determination (\( r^2 \)) of the method was >0.997 for each of the samples. The mean recoveries were satisfactory, ranging from 75 to 84% for all aflatoxins and the precisions, estimated by RSD%, were in the range of 7.2–9.7% (Table II).

### Table I

**Analytical Parameters of the UA-MSPD Method**

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>Slope ± SD</th>
<th>Intercept ± SD, ( r^2 )</th>
<th>LDR</th>
<th>( R ) (%)</th>
<th>LOQ</th>
<th>LOD</th>
<th>RSD (%) ( (n = 5) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB1</td>
<td>100,618 ± 8,051</td>
<td>297,705 ± 24,115, 0.9980</td>
<td>0.5–5.0</td>
<td>83.1</td>
<td>0.31</td>
<td>0.11</td>
<td>7.2, 8.9</td>
</tr>
<tr>
<td>AFB2</td>
<td>231,276 ± 17,340</td>
<td>124,541 ± 10,583, 0.9991</td>
<td>0.15–4.0</td>
<td>80.9</td>
<td>0.12</td>
<td>0.04</td>
<td>7.9, 9.0</td>
</tr>
<tr>
<td>AFG1</td>
<td>886,166 ± 76,753</td>
<td>211,408 ± 16,723, 0.9985</td>
<td>0.5–5.0</td>
<td>81.6</td>
<td>0.45</td>
<td>0.14</td>
<td>8.1, 9.5</td>
</tr>
<tr>
<td>AFG2</td>
<td>168,694 ± 12,034</td>
<td>87,789 ± 8,694, 0.9985</td>
<td>0.56–4.5</td>
<td>78.8</td>
<td>0.56</td>
<td>0.05</td>
<td>7.6, 8.8</td>
</tr>
</tbody>
</table>

All concentrations are based on ng g\(^{-1}\).

LDR, linear dynamic range; R, recovery.

### Table II

**Evaluation of the Recovery and Precision of UA-MSPD Method**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (ng g(^{-1}))</th>
<th>Precision (RSD%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB1</td>
<td>0.5</td>
<td>8.0</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.1</td>
<td>82</td>
</tr>
<tr>
<td>AFB2</td>
<td>0.5</td>
<td>7.9</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.3</td>
<td>81</td>
</tr>
<tr>
<td>AFG1</td>
<td>0.5</td>
<td>8.3</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8.6</td>
<td>79</td>
</tr>
<tr>
<td>AFG2</td>
<td>0.5</td>
<td>7.9</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.6</td>
<td>75</td>
</tr>
</tbody>
</table>

### Table III

**Determination of Aflatoxins in Different Rice Samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>AFB1</th>
<th>AFB2</th>
<th>AFG1</th>
<th>AFG2</th>
<th>Total aflatoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice 1</td>
<td>&lt;LOD</td>
<td>1.65</td>
<td>2.18</td>
<td>0.83</td>
<td>4.66</td>
</tr>
<tr>
<td>Rice 2</td>
<td>5.42</td>
<td>3.76</td>
<td>1.88</td>
<td>1.05</td>
<td>12.11</td>
</tr>
<tr>
<td>Rice 3</td>
<td>5.12</td>
<td>2.59</td>
<td>&lt;LOD</td>
<td>1.16</td>
<td>8.97</td>
</tr>
<tr>
<td>Rice 4</td>
<td>1.73</td>
<td>2.18</td>
<td>&lt;LOD</td>
<td>2.63</td>
<td>6.54</td>
</tr>
<tr>
<td>Rice 5</td>
<td>6.76</td>
<td>3.55</td>
<td>0.99</td>
<td>2.59</td>
<td>13.89</td>
</tr>
<tr>
<td>Rice 6</td>
<td>3.38</td>
<td>4.42</td>
<td>3.23</td>
<td>1.29</td>
<td>13.32</td>
</tr>
<tr>
<td>Rice 7</td>
<td>7.4</td>
<td>3.97</td>
<td>4.12</td>
<td>1.61</td>
<td>17.11</td>
</tr>
<tr>
<td>Rice 8</td>
<td>&lt;LOD</td>
<td>2.21</td>
<td>1.78</td>
<td>1.55</td>
<td>5.54</td>
</tr>
</tbody>
</table>

All concentrations are based on ng g\(^{-1}\).

<LOD, below the detection limit.

### Application to real samples

In order to evaluate the feasibility of the UA-MSPD method, eight different rice samples were obtained from different supermarkets and were analyzed under the optimal conditions. Table III shows the results of the three replicate analyses of each sample obtained by the current method. Four rice samples were positive for all aflatoxins. Figure 4 shows the chromatograms of a rice-positive real sample before and spiking. Table III shows the concentration of each aflatoxin in different rice samples.

### Determination of aflatoxins in FAPAS® T04145

In order to demonstrate the applicability of the proposed method for the analysis of complex matrixes, the concentration of aflatoxins was determined under optimum conditions in standard reference material (FAPAS®). Briefly, 0.5 g of FAPAS®...
standard was weighed and placed into a glass mortar, and then aflatoxins were determined by the general procedure. As it can be seen in Supplementary data, Table SIII, good correlation was achieved between the estimated content by the present method and reference material. Therefore, the UA-MSPD can be used as a trusty method for the extraction and determination of aflatoxins in real samples.

Conclusions
In this study, UA-MSPD in combination with HPLC-FLD has been developed as a new method for the extraction and quantification of AFB1, AFB2, AFG1 and AFG1 in rice samples. This method proposed the benefits of good detection limits and reproducibility, low cost, reduction in both the sample size and solvent consumption, lower waste generation and does not need any special instrumentation. In Supplementary data, Table SIV, we have tried to compare the present method with other methods, which are mentioned in the Introduction section. The comparison results showed that the proposed method is more sensitive (lower LODs) in some cases compared with other reported methods. Also, it has been shown that the RSDs of the present method are similar to those of other methods or are better. Hence, the analytical performance of this method is acceptable. Moreover, the UA-MSPD affords efficient clean-up compared with IACs; therefore, UA-MSPD can be an appropriate alternative. In addition, DLLME requires only small volume of low toxic extraction solvent for aflatoxins determination in contrast to UA-MSPD.

Supplementary data
Supplementary data are available at Journal of Chromatographic Science online.

Conflict of interest statement. The authors thank the financial support from Central Tehran Branch, Islamic Azad University through its "determination of aflatoxins in rice samples after ultrasound-assisted matrix solid-phase dispersion method", Project 2012/11/05. This article does not contain any studies with human or animal subjects.

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Figure 4. The chromatogram of rice real sample six analysis before and after spiking.
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