SIMULTANEOUS DETECTION OF TYPE A AND TYPE B TRICHOTHECENES IN CEREALS BY LIQUID CHROMATOGRAPHY COUPLED WITH ELECTROSPRAY IONIZATION QUADRUPOLE TIME OF FLIGHT MASS SPECTROMETRY (LC-ESI-QTOF-MS/MS)

Ala’ Yahya Sirhan a, Guan Huat Tan a & Richard C. S. Wong a
a Department of Chemistry, Faculty of Science, University Malaya, Lembah Pantai, Kuala Lumpur, Malaysia

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A new method based on Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique followed by liquid chromatography coupled with electrospray ionization quadrupole time of flight mass spectrometry (LC-ESI-QTOF-MS/MS) was developed for the determination of eight type-A and type-B trichothecenes in cereal samples. The quantification of the analytes was measured by performing low-energy collision induced tandem mass spectrometry (CID-MS) using the multiple reaction monitoring (MRM) mode. This method excludes the use of dispersive solid-phase extraction (dSPE) extraction cleanup step to reduce time and cost of analysis. Type-A trichothecenes were ionized in the positive ion mode and type-B trichothecenes in the negative ion mode. The recoveries of fortified cereal samples ranged from 61.9% to 110.9%, and the relative standard deviations (RSDs) were lower than the acceptable 12% in all the cases. The detection limits (S/N = 3) of type-A and type-B trichothecenes were 6.1–8.3 and 12.5–18.7 μg/kg, respectively.

**Keywords** cereals, liquid chromatography, mycotoxins, QTOFMS, QuEChERS, trichothecenes

**INTRODUCTION**

Trichothecenes belong to a group of mycotoxins, which are produced by the *Fusarium* molds. A total of 190 different structures have been discovered, all sharing a common tetracyclic, sesquiterpenoid 12, 13-epoxytrichothec-9-ene ring system. They fall into four distinct groups namely from A through D, and characterized by specific structural features. Type-A trichothecenes have a saturated carbon at C-8, characterized by an oxygen functional group and they include T-2 (T-2) and HT-2 toxins (HT-2), neosolaniol (NEO), and diacetoxyscirpenol (DAS). Type-B trichothecenes are functionalized by a
carbonyl group at the C-8 position and include deoxynivalenol (DON),
nivalenol (NIV), fusarenon X (FUSX), 3-acetyldeoxynivalenol (3-ADON),
15-acetyldeoxynivalenol (15-ADON), and their derivatives. Type-C trichothe-
cenes have a unique second epoxy group while the type-D trichothecenes
are distinguished by a macrocyclic structure.

The primary source of trichothecene contamination in food and feed-
stuff is cereal commodities (maize, oats, barley, and wheat) which have been
infected by the Fusarium fungi. While type-A and type-B trichothecenes are
commonly circulated by means of these crops, type-C and type-D trichothe-
cenes, although more toxic, rarely occur in food and feed. Type-A and
type-B trichothecenes exhibit acute toxicity and, when consumed, can result
in vomiting and loss of appetite; whereas, high concentrations of type-B
trichothecenes can cause chronic intoxication, leading to extensive
hemorrhage, and subsequent hematological toxicities. Type-A and Type-B
poisoning may also inhibit both in vitro and in vivo protein synthesis and
mitochondrial function, as well as manifest symptoms of immunosuppres-
sion at low concentrations. In particular, DAS exposure causes suppression
in the macrophage phagocytic function. Agricultural produce from Europe
are known to be free from type-A-trichothecenes and contain low concentra-
tions of DON, one of the most common mycotoxin pollutants. This is unlike
crops from elsewhere in which the presence of DON, as well as other Fusar-
ium mycotoxins, especially type-B trichothecenes, is common.

Because of their toxicity and frequent occurrence, several countries
have established legal regulations or recommendations for DON, HT-2
and T-2 toxins. The Food and Drug Administration (FDA) in the USA
recommends maximum contamination levels of 1000 µg/kg for cereal
products meant for human consumption, while the EU countries have
set standards of between 100 and 1000 µg of DON per kilogram for food,
and between 400 and 5000 µg/kg for feedstuff.\[1\]

In general, sample preparation is often the most important part in the
analysis of most mycotoxins and relies largely on the physiochemical proper-
ties of the commodities that are contaminated with mycotoxins. Commodi-
ties with high fat and pigment contents require more treatment. Traditional
strategies for the determination of mycotoxins in food commodities include
first extraction with a solvent or a mixture of organic solvents, followed by
re-constitution of the analyte and/or purification of the sample. Clean-up
methods are usually used for toxin isolation. The sensitivity and accuracy
of these methods depend on the ability to isolate selectively the toxins from
interfering matrix compounds present in natural samples prior to analysis.
These include solid-phase extraction (SPE) with a mixture of various solid
phases such as charcoal–alumina–Celite 545, cation exchange resin, alu-
mina–charcoal, in addition to multifunction column such as MycoSep
#225 and #227 columns.\[2\] Unfortunately, most of the methods applied
for extracting these toxins are either time-consuming, such as SPE, or expensive and matrix-dependent, such as multifunction column.

To simplify procedures for the sample preparation and to reduce the cost of analysis, the QuEChERS procedure was adopted, as it is a quick and economical way to extract food contaminants. The QuEChERS procedure is based on the extraction, liquid–liquid partition and dispersive-solid phase clean-up procedures. QuEChERS has many advantages over traditional techniques, such as high recovery, for wide polarity and volatility of analytes; high sample throughput and the use of small amounts of hazardous organic solvents; and finally the use of non-chlorinated solvents. In addition, it does not require expensive equipment, glassware such as vacuum manifolds and affords increased safety for workers in a laboratory environment. It is therefore a quick, easy, inexpensive, effective, rugged, and safe method.

Analytical methods for the determination of trichothecenes have been developed. The most common methods for isolating trichothecenes include gas chromatography (GC) with an electron capture detector (ECD),\(^2\) gas chromatography coupled with mass spectrometry (GC-MS),\(^3\) high performance liquid chromatography (HPLC) with a UV-Visible detector (UV), and HPLC with a fluorescence detector (FLD). Recently, liquid chromatography coupled with mass spectrometry (LC-MS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) have become common.\(^4\) It is easily amenable to the polar type-B trichothecenes and non-polar type-A trichothecenes without the need for derivatization. The current goal is to design a simple, fast, economical and effective means of extracting the sample using the QuEChERS method in addition to liquid chromatography coupled with electrospray ionization quadrupole time of flight mass spectrometry (LC-ESI-QTOF-MS/MS) for a reliable, precise and accurate quantification and confirmation of the type-A and type-B trichothecenes in cereal and cereal products.

**EXPERIMENTAL**

**Chemicals and Materials**

Trichothecenes standards of NIV, DON, 15-AcDON, 3-AcDON, FUS X, DAS, HT-2, and T-2 at 100 μg/kg concentration were obtained from R-BIOPHARM (Deisenhofen, Germany). The standard stock solutions were all dissolved in 2 mL of acetonitrile. Subsequently, daily working standard solutions were prepared by diluting the stock solutions in water containing 5 mM ammonium acetate and methanol containing 5 mM ammonium acetate 50:50 (% v/v). Glacial acetic acid (HPLC-grade) was purchased from Fisher Scientific (UK). Ammonium acetate, HPLC-grade, was purchased from Fluka (Darmstadt, Germany). Sodium chloride was purchased from...
BDH PROLABO (EC Countries). Anhydrous magnesium sulfate (MgSO₄) for QuEChERS was purchased from Agilent Technologies (USA). Water was purified by reverse osmosis with an electrodeionization (EDI) system (Maxima Ultra Pure Water, England). Methanol and acetonitrile, HPLC-grade, were obtained from Merck (Darmstadt, Germany). A nonsterile PTFE Syringe Filter with disposable membrane filter (0.22 μm) was purchased from Membrane Solutions (USA).

Food Samples

In the month of December 2010, 1–2 kg each of 25 samples of cereals and cereal products (wheat, wheat based noodles, rice, rice based noodles, and corn) were randomly obtained from groceries and stores in Kuala Lumpur, Malaysia. The samples were stored in the dark at room temperature (20–25°C). The samples were ground and mixed at room temperature for 10 min until a fine and homogeneous powdered material was obtained. The powdered samples were then stored in plastic bags at 4°C in a refrigerator prior to analysis.

Sample Preparation

Cereal samples were prepared using a previously published procedure[5] with the following modifications.

Step I
A thoroughly homogenized cereal sample (1.0 g) was weighed in a polypropylene centrifuge tube (15 mL).

Sample recovery was performed with (1.0 g) of the blank wheat samples with two different fortification levels; 0.5 mL of trichothecene mixed standards were spiked at 100.0 and 500.0 μg/kg of the standard mix. The spiked samples were left overnight in the dark at room temperature to allow the solvent to evaporate and for aflatoxin absorption into the matrix. Then, they were extracted via the following steps (II to IV).

Step II
A 3.0 mL sample of 79:20:1 (% v/v) acetonitrile/water/acetic acid mixture was added and the centrifuge tube was shaken for 1 min to ensure that the solvent has mixed thoroughly with the entire sample, for complete extraction of the analyte.

Step III
A 0.8 g sample of anhydrous MgSO₄ and 0.2 g of NaCl were added into the mixture and the shaking procedure was repeated for 1 min to facilitate
the extraction and partitioning of the eight trichothecenes into the organic layer.

**Step IV**
The extract was centrifuged for 5 min at 4000 rpm, and 0.5 mL of the upper organic layer was filtered through a 0.22 μm nylon syringe filter prior to LC-ESI-QTOF-MS/MS analysis.

**Analytical Procedure**

The quantification of the analytes was measured by performing low-energy collision induced tandem mass spectrometry (CID-MS/MS) using the multiple reaction monitoring (MRM) mode. The quantification of the trichothecenes were performed using an Agilent 6530 Q-TOF-MS/MS spectrometer coupled with an Agilent 1200 Series HPLC system. The HPLC system consists of a vacuum degasser (G1379B) with a thermostated autosampler (G1330B) and binary pump (G1312B), and a thermostated column compartment (G1316B). The QTOF-MS/MS instrument was equipped with an ESI source and the Agilent jet stream dual nebulizer.

**Instrument Conditions**
The injection volume was fixed at 5 μL for the samples and standards. The sample extract was injected into a ZORBAX Eclipse XBD-C18, 2.1 mm × 100 mm, 1.8 μm (P.N. 928700-902) column at a flow rate of 0.2 mL/min running with 70% and 30% mobile phase A and B, respectively. Mobile phase A consists of 1% acetic acid and 5 mM ammonium acetate in water and mobile phase B consisted of 1% acetic acid and 5 mM ammonium acetate in methanol. The gradient was changed to 80% mobile phase B over 10 min, and then maintained for 3 min. After 13 min of run time, the gradient was returned to 30% mobile phase B over 1 min. The column was washed for 6 min with water and conditioned for 4 min before the next injection. The mass spectrometer was operated in the positive and negative ESI mode. In this method, there were two periods with different polarities (0–10 min negative; 10–14 min positive) were employed. The optimum QTOF-MS/MS operational conditions are as follows: the drying gas and sheath gas temperatures were set at 150°C and 350°C, respectively, while the drying gas and sheath gas flow rates were set at 5 L/min and 12 L/min, respectively. The nebulizer pressure was set at 25 psi and the nozzle voltage was set at 0 V. Finally, the capillary voltage was set at 3500 V, while the fragmentor voltage was set at −60 V in the negative mode and +160 V in the positive mode.
RESULTS AND DISCUSSION

QuEChERS Method Development

The preparation of the extract has a crucial impact on the accuracy of the results and there are several factors that can affect performance in the original QuEChERS method such as the composition of extraction solvent, the type and amount of the drying agents, extraction time, and the dilution factor. All of these factors were taken into consideration and then optimized. The extraction solvent was found to be the most important factor and it heavily affects the extraction efficiency. However, the physicochemical properties of type-A and type-B trichothecenes are different, with the type-A trichothecenes being relatively nonpolar compounds and the type-B trichothecenes are polar compounds. Hence, the selection of a suitable extraction solvent for both types of trichothecenes is difficult. From a review of the literature, six different extraction mixtures were tested in blank wheat samples spiked with 500 µg/L of the trichothecenes standards. The results using all these extraction solvents were then compared.

1. Extraction solvent 1: A mixture containing 79:20:1 (%, v/v) acetonitrile/water/acetic acid was employed.\(^6\)
2. Extraction solvent 2: A mixture of 57:42.5:0.5 (%, v/v) acetonitrile/water/acetic acid was employed.
3. Extraction solvent 3: A mixture of 79:20:1 (%, v/v) methanol/water/acetic acid was employed.
4. Extraction solvent 4: A mixture of 85:15 (%, v/v) methanol/acetonitrile was employed.\(^7\)
5. Extraction solvent 5: A mixture of 20/80 (%, v/v) methanol/acetonitrile was employed.
6. Extraction solvent 6: A mixture of 80/20 (%, v/v) methanol/water was employed.

The results as shown in Table 1 demonstrate that 57:42.5:0.5 (%, v/v) acetonitrile/water/acetic acid and 85:15 (%, v/v) methanol/acetonitrile combinations present good recoveries for relatively nonpolar type-A trichothecenes, but not with the polar type-B trichothecenes such as NIV and DON. However, 79:20:1 (%, v/v) acetonitrile/water/acetic acid offers recoveries that are satisfactory for polar and non-polar analytes. In addition, it gave recoveries fulfilling the EU Commission Directive 2006/401/EC\(^8\) for analysis of mycotoxins in food samples. Therefore, mixtures of 79:20:1 (%, v/v) acetonitrile/water/acetic acid were applied in this study.
Optimization of the Liquid Chromatography (LC) Conditions

In addition to improving resolution in the chromatographic system, the selection of the mobile phase based on consideration of the ionization efficiency by the ESI source, it is also important to obtain good resolution and high sensitivity. By using the ESI source, solvents can greatly influence the generation of small droplets, which leads to increased evaporation of solvents and so enhance the sensitivity. The use of methanol instead of acetonitrile in the solvent mixtures resulted in better ionization and higher intensities for all analytes, probably due to the proton affinity of some of the isomeric forms of acetonitrile generated, especially for DON, T-2, and HT-2.

The effects of acetic acid, formic acid, ammonium acetate, and ammonium formate on the analyte response were tested by using various combinations. After addition of either ammonium acetate or acetic acid, no significant increase in sensitivity was observed. On the other hand, the addition of a mixture of acetic acid (1%) and ammonium acetate to the mobile phase, led to a significant increase in the response for all the targeted analytes, due to an improvement in the peak shape and better ionization. As for the choice between using a mixture of ammonium acetate/acetic acid (1%) and ammonium formate/formic acid (1%), the results indicated that when ammonium formate was chosen, the ionization of all the selected analytes was significantly reduced. Whereas, the use of ammonium acetate at a concentration of 5 mM and acetic acid (1%), was found to be sufficient to generate $[M+\text{NH}_4]^+$ or $[M+\text{Na}]^+$ ions in the positive mode.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Mycotoxin$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>79:20:1 (% v/v) acetonitrile/water/acetic acid</td>
<td>90 ± 7.7</td>
</tr>
<tr>
<td>57:42:5:0.5 (% v/v) acetonitrile/water/acetic acid</td>
<td>40 ± 10.7</td>
</tr>
<tr>
<td>79:20:1 (% v/v) methanol/water/acetic acid</td>
<td>-b</td>
</tr>
<tr>
<td>85:15 (% v/v) methanol/acetonitrile</td>
<td>57 ± 5.6</td>
</tr>
<tr>
<td>20/80 (% v/v) methanol/acetonitrile</td>
<td>85 ± 8.2</td>
</tr>
<tr>
<td>80/20 (% v/v) methanol/water</td>
<td>-b</td>
</tr>
</tbody>
</table>

$^a$Recovery ± RSD (%).
$^b$Mean recovery value is <25%.

**TABLE 1** Assessment of Different Extraction Solvents in Wheat (1g) Samples Spiked at 500 μg/kg Level (n = 5)
for the type A-trichothecenes. In the negative mode, either \([M + Br]^-\) or \([M + CH_3COO]^-\) ions can be generated for the type B-trichothecenes, whereas at higher concentrations (\(>10\,\text{mM}\)) ionization suppression was observed. The presence of cation and anion impurities in the HPLC solvents can explain for this phenomenon, and furthermore, sodium ions are more abundant than ammonium impurities in methanol.

For the gradient elution conditions, the main task was to determine the composition of solvent A and solvent B as the initial mobile phase. Several initial compositions of the mobile phase were studied, ranging from 5\% to 40\% of solvent B. The results showed that good peak shapes and high sensitivity were achieved with the decrease of solvent B content in the initial composition. However, when the initial gradient started at 40\% of solvent B, overlapping peaks were obtained for DON and NIV. Considering the combined factors of separation efficiency, run time and sensitivity, the use of 30\% of solvent B was selected as the initial composition of the mobile phase (Figure 1). In the case of 3-ADON and 15-ADON, in addition to having the same MS/MS spectrum, separation under these conditions were not achieved. Hence, both isomers were determined as total ADON.\(^9\)

Other parameters such as flow rate, injection volume, and column temperature were optimized and the following parameters were selected as optimum conditions: 0.20 mL/min as flow rate, 40°C as column temperature, and 5 \(\mu\)L as the injection volume.

**Optimization of the ESI Parameters**

Both the positive and negative modes were investigated for ion acquisition in the MS. NIV, DON, FUS X, and ADON showed higher signal

![FIGURE 1](https://example.com/figure1.png)  
**FIGURE 1** Extract ion chromatogram (EIC) (LC/QTOF-MS/MS) of separation of a trichothecenes mixture solution containing 600 μg/kg for each toxin. Vertical line illustrates change of ionization polarities from negative to positive (10 min). (Color figure available online.)
intensities in the negative ionization mode, whereas DAS and T-2 undergo better ionization in the positive mode. Detection of HT-2 can be obtained in both positive and negative ion mode, with a slight increase in sensitivity and higher stability in the positive ion mode compared to the negative mode.

The instrumental parameters (drying and sheath gas flow rates and temperatures, fragmentor and capillary voltage) were optimized to provide the best possible sensitivity. However, it was found from a previous study that the effects of all these parameters did not significantly affect the signal from the analytes. However, the fragmentor voltage, which played an important role in both the sensitivity and fragmentation, did influence the sensitivity. The fragmentor voltage (cone voltage or orifice voltage) is the difference between the voltage applied to the cone and that applied to the skimmer. Increasing the potential difference between the two would increase the kinetic energy and, thus, the velocity of the charged analyte droplets with more ions being transferred toward the skimmer resulting in a significant enhancement of the transmission efficiency and sensitivity. For this reason, the fragmentor voltage value was studied in the range from 20 to 400 V under optimized source conditions.

For type-B trichothecenes, fragmentor voltages greater than 60 V led to extensive fragmentation even for the reference masses, whereas for type-A trichothecenes, extensive fragmentation was observed at even higher fragmentor voltages ($\geq 180$ V). Voltage values in the region of 160 V provided minimal fragmentation and adequate sensitivity for quantification for type-A trichothecenes, while voltage values of approximately 60 V provided minimal fragmentation and adequate sensitivity for quantification for type-B trichothecenes. The presence of the sodium or ammonium adducts were observed as a base peak for type-A trichothecenes and the bromo adduct for type-B trichothecenes (Figure 2). However, the protonated molecules are also present in the mass spectrum and they can be used for quantification.

**Selection of Product Ions**

The selection of product ions was carried out by varying the collision energy between 5 and 45 V, at increments of 5 V. Table 2 shows the final selection of the product ions against the optimal collision energy. Two product ions were selected, the more intense as the quantifier ion and the other as the qualifier ion. Furthermore, the selection of the product ions was carried out for confirmation of the identity of the analyte, which can also be confirmed by both the analyte retention time and the accurate mass by using a maximum mass error of 5 ppm of the analyte precursor ion resulting from the TOF mass analyzer.
Method Validation

This was an in-house validated method, in terms of linearity, accuracy, intra-day precision, inter-day precision, limit of detection (LOD), and limit of quantification (LQ).

**TABLE 2** MS/MS Parameters for Detection of the Measured Trichotheccenes in the Multiple Reaction Mode (MRM)

<table>
<thead>
<tr>
<th>Mxotoxin</th>
<th>Molecular ion</th>
<th>Retention time (min)</th>
<th>Precursor ion (m/z)</th>
<th>Collision energy (V)</th>
<th>Product ions&lt;sup&gt;a&lt;/sup&gt; (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIV</td>
<td>[M+Br]&lt;sup&gt;-&lt;/sup&gt;</td>
<td>3.3</td>
<td>391.0582</td>
<td>5</td>
<td>281.1013/311.1125</td>
</tr>
<tr>
<td>DON</td>
<td>[M+Br]&lt;sup&gt;-&lt;/sup&gt;</td>
<td>4.4</td>
<td>375.0446</td>
<td>5</td>
<td>295.1171/265.1082</td>
</tr>
<tr>
<td>FUS X</td>
<td>[M+Br]&lt;sup&gt;-&lt;/sup&gt;</td>
<td>6.3</td>
<td>433.0498</td>
<td>30</td>
<td>263.0928/205.0841</td>
</tr>
<tr>
<td>ADON</td>
<td>[M+Br]&lt;sup&gt;-&lt;/sup&gt;</td>
<td>9.2</td>
<td>417.0562</td>
<td>10</td>
<td>337.1297/307.1185</td>
</tr>
<tr>
<td>DAS</td>
<td>[M+Na]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>11.1</td>
<td>389.1589</td>
<td>15</td>
<td>278.9576/210.1467</td>
</tr>
<tr>
<td>HT-2</td>
<td>[M+Na]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>11.7</td>
<td>447.2051</td>
<td>15</td>
<td>297.1239/251.0834</td>
</tr>
<tr>
<td>T-2</td>
<td>[M+Na]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>12.4</td>
<td>489.2159</td>
<td>15</td>
<td>238.0998/305.2479</td>
</tr>
</tbody>
</table>

<sup>a</sup>Numerical values are given in the order quantifier/qualifier.
of quantification (LOQ). The linearity was tested using a standard solution of the trichothecenes in the concentration range of 75 and 200 μg/L. Table 3 shows that good linear relationships with correlation coefficients greater than 0.97 for all targeted analytes were obtained. Calibrations with standard solutions were used for quantitation, because moderate signal suppression was noticeable for all the analytes. Furthermore, the ANOVA test, did not give any significant difference at p = 0.05.

The accuracy was tested by the determination of the recoveries of the trichothecenes in clean wheat, wheat products (wheat-based noodles), rice, rice products (rice-based noodles), and corn samples spiked at 100.0 and 500.0 μg/kg of the trichothecenes standards and were analyzed in triplicates (Table 4). The recoveries obtained ranged from 61.9% to 110.97%, with a relative standard deviation (RSD) of less than 12%. The recoveries for relatively nonpolar trichothecenes (DAS and HT-2) were slightly more significant than the polar analytes (NIV and DON).

The sensitivity was determined by estimating the limit of detection (LOD) and limit of quantification (LOQ). LODs and LOQs were estimated experimentally as the lowest concentration giving a response of three- and ten-times, respectively, the baseline noise given by the software, obtained from mycotoxin-free samples. The LOD of type-A and type-B trichothecenes were 6.1–8.3 and 12.5–18.7 μg/kg, respectively (see the details in Table 3).

Intra-day precision was evaluated by assaying five replicates of the same sample at a spiked level of 500 μg/L trichothecenes on the same day. For the inter-day precision, five replicates of the same sample at a spiked level of 500 μg/L trichothecenes were analyzed on three consecutive days. The intra-day precision and inter-day precision were calculated and tabulated in Table 3. The intra-day precision (n = 5) are between 1.1 and 6.7%, while the inter-day variation (n = 15) values are between 4.8 and 14.3%. These

### TABLE 3
Mean of Recoveries and RSDs (n = 5) of Trichothecenes Spiked into Blank Wheat, Wheat Product, Rice, Rice Product and Corn Samples at Two Spiking Levels

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Spiking level (μg/kg)</th>
<th>NIV</th>
<th>DON</th>
<th>FUS X</th>
<th>ADON</th>
<th>DAS</th>
<th>HT2</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat 100</td>
<td>77.2 ± 10.3 77.4 ± 8.4 105.9 ± 6.2 110.1 ± 8.1 95.3 ± 9.2 119.9 ± 10.8 109.0 ± 7.1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wheat 500</td>
<td>80.7 ± 4.5  82.4 ± 4.9 95.0 ± 9.9 107.9 ± 4.0 106.6 ± 5.0 106.8 ± 5.5 95.3 ± 4.4</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wheat product</td>
<td>78.1 ± 9.1  76.4 ± 8.7 99.4 ± 4.5 105.3 ± 4.3 107.0 ± 7.8 90.5 ± 2.8 110.1 ± 9.8</td>
<td></td>
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<tr>
<td>Rice 100</td>
<td>77.3 ± 9.4  76.9 ± 8.5 116.8 ± 9.6 109.5 ± 6.3 94.4 ± 7.1 99.6 ± 7.3 90.6 ± 8.3</td>
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</tr>
<tr>
<td>Rice 500</td>
<td>79.9 ± 9.4  72.5 ± 6.2 104.4 ± 6.4 108.0 ± 4.5 106.4 ± 9.2 103.6 ± 4.5 103.6 ± 9.4</td>
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</tr>
<tr>
<td>Rice product</td>
<td>84.7 ± 6.9  70.2 ± 8.6 82.8 ± 7.4 104.8 ± 8.2 90.0 ± 9.8 86.1 ± 7.2 96.8 ± 10.2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Corn 100</td>
<td>61.9 ± 8.5  73.3 ± 9.4 96.5 ± 10.8 92.7 ± 11.1 74.0 ± 7.1 71.6 ± 10.0 84.9 ± 10.0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Corn 500</td>
<td>65.7 ± 4.2  78.8 ± 8.5 99.1 ± 5.0 95.2 ± 7.9 80.6 ± 9.4 87.2 ± 5.7 89.3 ± 5.4</td>
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</table>
values determined are lower than the acceptable maximum of 15%, confirming the good reproducibility and repeatability of this technique.

Considering the data for method validation, the current LC-ESI-CID-MS/MS analysis measured with a hybrid QTOF-instrument and sample preparation procedures employed can be regarded as selective, precise, and robust.

**CONCLUSION**

A simple, rapid, and confirmatory method has been developed for the determination of type-A and type-B trichothecenes in cereals. The use of QTOF-MS/MS uniquely offers the possibility of providing accurate mass data and can generate structural information of the analytes with minimal sample treatment and without derivatization. The sensitivity of the instrument could be significantly enhanced by optimizing the chromatographic conditions and the fragmentor voltage in the ESI interface. Extensive and expensive clean-up procedures could be replaced by adopting the QuEChERS procedure without prior dSPE step. The extraction solvent was found to be the most important factor and strongly affects the extraction efficiency. Excellent linearity, high recoveries, acceptable repeatability, and reproducibility with the LOQ values lower than the stipulated MRL values were achieved indicating the suitability of the proposed method for the determination of trichothecenes in cereals.

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REFERENCES