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Swift and Enhanced-Sensitive Analytical Method for Determination of Fumonisins FB1 and FB2 in Egyptian Oilseeds Using LC-MS/MS.

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Abstract

Fumonisins (FBs) are harmful carcinogenic natural toxins produced by fungi from *Fusarium species* whereas found in oilseeds crops. Fumonisin B1 (FB1) and Fumonisin B2 (FB2) are the most serious natural food contaminants among the several known Fumonisin. The main purpose of this study is to establish an accredited rapid and ultra-sensitive method for the investigation of FB1 and FB2 in Egyptian oilseed. LC-MS/MS with the positive-ion electrospray ionization (+ESI) mode has been employed for this investigation. The limits of quantification (LOQ) were 3.9 µg/Kg for FB1 and 2.3 µg/Kg for FB2. The limits of detection (LOD) were 1.2, and 0.7 µg/Kg for FB1 and FB2 respectively with 21% expanded uncertainty. The test method has been used to review six governorates of Egypt on corn, white corn, soybean, sesame, and sunflower seeds. For corn, the contamination levels of total FB1 and FB2 were varied between <LOQ to 10034.9 µg/Kg, while in white corn was from <LOQ to 7571 µg/Kg. The total FB1 and FB2 were from <LOQ to 321.9 µg/Kg in soybean samples. Whereas sesame and sunflower seeds were devoid of FB1 and FB2.

Keywords: Fumonisin,FB1,FB2, LC-MS/MS, method determination, Egyptian oilseed.

1. Introduction

Mycotoxins, which are toxic compounds that are produced as secondary metabolites by several filamentous fungi, are recognized as food contaminants worldwide, causing significant economic losses in agriculture, and posing public health risks [1,2,3,4,5,6,7,8]. Fumonisins are compounds of mycotoxins that were produced by Fusarium fungi species, primarily Fusarium verticillioides, and F. proliferatum [9]. Fumonisin B1 (FB1) and fumonisin B2 (FB2) have been considered one of the most serious natural food contaminants among the several known fumonisins [10]. In terms of carcinogenicity studies, International Agency for Research on Cancer has classified FB1 as a possible human carcinogen (Group 2B) [11,12,13]. The European Union (EU) set the maximum permissible levels depending on the sum of two types FB1 and FB2 for unprocessed maize (4000 µg/Kg) while maize prepared to direct human consumption (1000 µg/Kg) moreover maize-based breakfast cereals, and maize-based snacks (800 µg/Kg), processed maize-based foods, and baby food for young children and infants also (200 µg/kg) [14]. High levels of fumonisins were found mainly in cereals grown in the tropical and subtropical regions [15,16,17]. Corn (yellow corn) and maize(white corn) are the most common cereals grown in our world, and it is present in significant amounts in the diet of the population, either directly or through processed products such as cakes, bread, biscuits, and Snacks [18]. Fumonisin has been discovered as contaminants in a variety of corn products, including precooked corn flakes, roasted corn flour, corn grits, white corn grits, and cornmeal [19]. The presence of fumonisins in maize and corn can be influenced by climatic factors and physical damage, as well as insufficient drying, transport, and storage [1,20,18,21].Egypt imported about (10,000,000 and 4,750,000 and 95,000) tones in 2020 from corn, Soybean, and sunflower seeds respectively [22,23,24]. Several LC-MS/MS methods have been optimized for

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fumonisin determination in various food matrices [25,26,27]. Various sample treatment approaches have been reported with solid-phase extraction (e.g. immuneaffinity columns, and C18 columns) [28,29,30,31,32]. Clean-up steps take a long time and may cause a loss of part of the target, which increases the cost of the method. On the other hand, studies based on dilution and injection techniques without clean-up steps showed fast, efficient, and reliable results [26,33,25,34]. Many investigations for the determination of the Fumonisin levels in various countries have been conducted [35,36,37].The last reported study on Fumonisin levels in Egypt has been performed in 2009[38] which resulted in contamination and regulation violation of all samples with Fumonisin, although the a high risk of Fumonisin no more studies have been conducted since there.

This study aimed to introduce an optimized assay protocol for the concurrent determination of FB1 and FB2 in oilseeds using LC-MS/MS. A dilute and shoot analysis approach will be employed to efficiently extract the studied compounds along with the shortest possible sample processing features. Validation of the proposed method as per EU guidelines covering LOD, LOQ, linearity, working range, percentage recovery, precision, and measurement uncertainty. Method's practicality testing through application to PT and real samples. Highlighting the level of contamination by FB1 and FB2 in Egyptian oilseeds.

2. Material and Method

2.1. Chemicals and reagents

All solvents and chemicals that have been used in the study were of the analytical grade. Deionized water was generated by Milli-Q A10. Acetonitrile (ACN) and methanol (MeOH) were purchased from Merck, 99.9% purity. Formic acid (Riedel-de Häen, 98-100.5%). Ammonia solution (Riedel-de Häen, 30-33%). The reference standard mixture of FB1 and FB2 has been purchased from Sigma-Aldrich (MilliporeSigma Canada Ltd).

2.2. Instruments

In this study, we employed an LC-MS/MS system consisting of a Thermoscientific Vanquish HPLC system coupled with a tandem mass spectrometer system TSQ ALTIS-MS/MS instrument. We used 50 ml polypropylene centrifuge falcon tubes with screw caps (Supelco, Bellefonte, USA), Polytetrafluoroethylene (PTFE) membrane syringe filter with 0.2 µm pore size, Geno/Grinder SPEX® sample preparation shaker device (SPEX, USA)), and lab mega-fugue centrifuge (HERMLE, Z32 HK). Also, we needed to use injection glass vials with Teflon coated caps (Dotmed, USA), volumetric flasks (10, 25, and 50 ml), graduated glass pipettes (5, 10, and 20 ml), bottle top dispenser (5–50 ml) (Hirschman, Germany), fixed micropipette 50 µl and variable micropipettes (2–20 µl) and (200–1000 µl) (Eppendorf, Germany), benchtop pH-meter. Analytical balance and precision balance (Mettler-Toledo, Selangor), water purification system MilliQ UF-Plus system (Millipore, Germany) had been used also.

2.3. Standard solutions

Stock solution mixture of FB1 and FB2 (50 mg/L) dissolved in acetonitrile/water (1:1, v/v) has been purchased from Sigma-Aldrich (MilliporeSigma Canada Ltd) and kept in a freezer at -20 ˚C. Working solutions mixture (1 mg/L) has been prepared by diluting the fitting volume of stock solutions in the volumetric flask with acetonitrile/water (1:1, v/v) and stored in small portions (1 ml) in small vials and kept in a freezer at +4 ˚C.

2.4. Sampling

A total of 300 samples, 6o from each commodity, corn, white corn, soybeans, sesame, and sunflower seeds were collected from different seed retailers, fodder shops, and fields in various governorates of Egypt (Cairo, AL-Giza Al-Qalyubia, Al-Gharbia, Al-Bahira, and Al-Menoufia) in 2020 and 2021, About one hundred and fifty samples per year.

2.5. Sample handling and storage

All collected samples (1 kg of each) were kept in polyethylene plastic bags at +21 ˚C ±3 ˚C until analysis. Before analysis, all samples had been ground well by using a commercial milling machine to achieve complete homogeneity and ideal dissemination of fumonisins in potentially contaminated samples.

2.6. Validation and accreditation

The test method has been validated to characterize the analytical parameters and confirm that the test method being considered has performance capabilities that were consistent with the application's requirements according to Eurachem guidelines [39]. Also, The Finish Accreditation Service (FINAS) has granted this method international accreditation for meeting the requirements of the ISO/IEC 17025:2017 standard.

2.7. Sample preparation and processing

Five grams of a ground sample have been transferred into a polytetrafluoroethylene(PFTE) tube 50 ml mixed with 20 ml ACN: Water: Formic acid (79:20:1, v/v/v). Tubes were shocked by a vertical mechanical shaker (GENO) at 700 RPM for 20 min. After that, all tubes were put in the centrifuge for 5 min at 4000 RPM under cooling conditions. The obtained supernatants have been filtered through 0.20 µm PTFE acrodisc into a 2 mL vial and directly injected into LC-MS/MS system.

2.8. Matrix standard

Matrix matched standard is used to correct for matrix effect. Prepare matrix-matched standards by diluting blank matrix extract with a standard solution. To reduce the number of errors caused by matrix-induced effects during chromatography, it is best to choose similar commodities. It is better not to dilute the matrix by more than 20% to avoid errors caused by differences in the matrix-induced enhancement effect between sample extract and matrix standard.

3. Results and Discussion

3.1. Optimization and Chromatographic conditions

3.1.1. Extraction solvent optimization

Many extraction mixtures with different ratios have been used through extraction step, In Gazzotti et al. 2011 the extraction solvent was Methanol: water (80:20,v/v)[40]. In Bryła et al. 2016 the extraction solvent was ACN/MeOH/water (25:25:50, v/v/v [41].These methods are based on clean-up by different solid-phase-extraction (SPE), these take a long time and may cause loss of part of the target compound, need well training person, and increases the cost of the analysis. In the present study, we needn't make clean-up by SPE and that makes us save a lot of time. Also, these previous methods take a long time to separate the target compound on the LC-MS/MS and the run time is usually high compared to the present method which needs five minutes to separate FB1 and FB2, which reduces the time of using LC-MS/MS and therefore reducing the cost of the method. However, the recovery and the sensitivity of the present method are higher than the previous methods, which makes the present method more effective use from these methods.

Andrade et al., 2017 the extraction solvent was ACN: Water: Acetic acid (80:20:0.1, v/v/v) with shaking for 45 min using the internal stander in the injection on LC-MS/MS [26]. Also, in Franco et al. 2018 the extraction solvent was ACN: Water: Acetic acid (80:20:0.1, v/v/v) with shaking for 90 min using the internal stander in the injection on LC-MS/MS [33], In Hu et al., 2019 the extraction solvent was ACN: Water: Acetic acid (70:29:1, v/v/v) with shaking for 30 minutes and using the lapelled internal stander in the injection on LC-MS/MS[25]. While the extraction solvent in de Matos et al., 2021 was ACN: Water: formic acid (75:24:1, v/v/v) with shaking for 20 min using the internal stander in the injection on LC-MS/MS [34]. These previous methods are based on the Dilute-and-Shoot technique with using the lapelled internal stander in the injection on LC-MS/MS. Lapelled internal stander increases the analysis efficiency but increases the cost also. In the present study, there are many tests were conducted to select the most efficient solvent for extracting fumonisin from seeds based on low cost with safer handling for analysts by following the "Dilute-and-Shoot" technique but without using the lapelled internal stander in the injection on LC-MS/MS and replace it by the matrix-matched standard to correct the matrix effect.

We tested the extracting solvent of the previous methods A [ACN: Water: Acetic acid (80:20:0.1, v/v/v)], B [ACN: Water: Acetic acid (70:29:1, v/v/v)], C [ACN: Water: Acetic acid (80:19:1, v/v/v)], and finally D which was the extraction solvent C with some modification by replacing the Acetic acid with Formic acid D [ACN: Water: Formic acid (79:20: 1, v/v/v)] with shaking for 20 minutes without using the lapelled internal stander. The best extraction solvent recovery was the extraction solvent D was ranged from 90.1% to 113.6 %, Also the matrx recovery were mor than 85 %, which corresponded to the European Commission regulation [42]. The high percentage of the formic acid and ACN increasing the ability of the extraction solvent to extract the fumonisins from the ground seeds, also the formic acid helping to avoid the interaction between the active site in the glass surface and fumonisins.

3.1.2. LC Condition

In previous studies, there were many columns used to separate FB1 and FB2 in LC-MS/MS with different run Times. In Gazzotti et al. 2011 the column was Waters XTerra MS C18 column (5 µm, 2.15 ×150 mm) with a run time of 17 min [40]; In Bryła et al. 2016 the column was Kinetex PFP 100 mm ×2.1 mm, 2.6 mm (Phenomenex, Torrance, CA, USA) column with run time 55 min [41]; In Andrade et al., 2017 the column was Gemini C18 analytical column (150 × 4.6 mm, 5 m) with run time 12 min[26]; in Franco et al. 2018 the column was BEH C18 column (2.1 × 50 mm,

1.7 µm) with run time 10 min [33]; in Hu et al., 2019 the Phenomenex Luna C18 column (150 mm × 2 mm, 3 µm) with run time 15 min [25]; While in de Matos et al., 2021 the column was BEH C18 column (100 mm × 2.1 mm i.d., 1.7 µm) with a run time 4.5 min [34].

In the present study, LC analysis has been performed on three different columns A:(Acclaim™ RSLC Polar Advantage 120 II Å column (75 mm × 3.0 mm, 3 µm)), B: (Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 µm), and C:(Poroshell 120 EC- C18 column (50 mm × 3 mm,2.7 µm), at a flow rate of 0.3 mL/min. The column temperature has been set at 40◦C, and the resolutions, separation efficiencies, and the peak shapes of the target analytes on these columns had been compared. The mobile phases were 10 mmol/L ammonium formate water solution (A) with methanol (B) and the FB1 and FB2 were eluted with an isocratic elution program. The separation of analytes FB1 and FB2 in Acclaim column (A) was about 1.5 min between FB1 and FB2 but the sensitivity was less than in the other columns in FB1 and FB2. Also, the symmetric of the peak cheap wasn't good for the FB2 figure1. In the Eclipse column (B) the separation was less than in the other columns it was about 0.44 min between FB1 and FB2. The sensitivity was high compared to the Acclaim column but less than the poroshell column. The symmetric of the peak cheap wasn't good for both of analyts FB1 and FB2 and the runtime was more than the other columns it was 10 min figure 2. In the poroshell column(C) the run time was 5 min and the separation was more than the other columns it was about 2.45 min between FB1 and FB2. Also, the sensitivity was greater than the other columns, and the peak cheap's symmetric was great for both FB1 and FB2 figure 3. The run time in the present study was less than all the previous studies except de Matos et al., 2021 [34] it was so closed.

The condition for the finally selected Poroshell column(C). It was programmed as follows: 3.0 min, 40% A2, and 60 % B2. The injection volume was 2 µL. The runtime was 5 min the retention time (RT) of FB1 was 1.57 while FB2 was 4.02 as shown in figure 3. To avoid the contamination of mass spectrometer. The detection and quantification of the target compound have been carried out using's ALTIS-MS/MS instrument, connected to HPLC via a turbo spray ion drive electrospray ionization (ESI) interface. The ESI was operated in positive mode ESI (+) by using the following operating parameters:

Table 1: LC-MS/MS mas' condition for determining FB1 and FB2.

Mas Condition	
Ion Source type (IS)	H-ESI positive mode
Spray Voltage	+ 4000 V
Sheath Gas	50
Aux Gas	15
Sweep Gas	1
Ion Transfer Tub Temp (°C)	350
Vaporizer Temp (°C)	400
Polarity	Positive
Resolution (FWHM)	1.2
CID Gas (m Torr)	1.5
Chromatographic Peak Width (sec)	6

In previous studies, they used the precursor ion for FB1 722 (m/z) with two product ions 334 and 352 (m/z). While FB2 the precursor ion for FB2 was 706 (m/z) with two product ions 336 and 318 (m/z) [40,43]. In the present study we used 722.8(m/z) as the precursor ion for FB1 with three product ions (334.2, 352.5, and 704.5) (m/z). According to the sensitivity of product ions in the real sample, we selected 334.2 (m/z) as a primary quantifier it has high sensitivity compared to the other product ions. While 352.5 and 704.5 (m/z) were used as a secondary to qualified figure 4. Also, the precursor ion for FB2 was 706.7(m/z) with two Product ions 336.1 (m/z) used as a primary quantifier as it has high sensitivity compared to 318.1 (m/z) which is used as a secondary to qualified figure 5.

TraceFinder™ 5.1 software was used for instrument control and data processing and method acquisition.

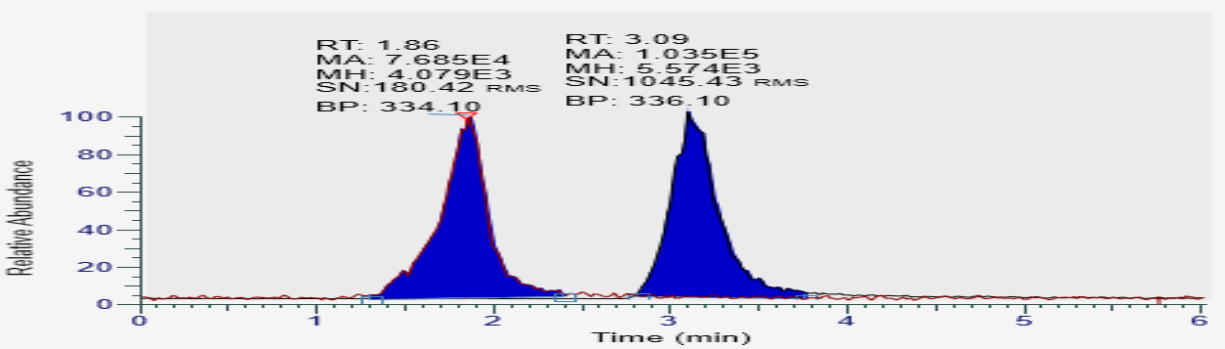

Figure 1:Extracted ion chromatogram of FB1 and FB2 by Acclaim column (A).

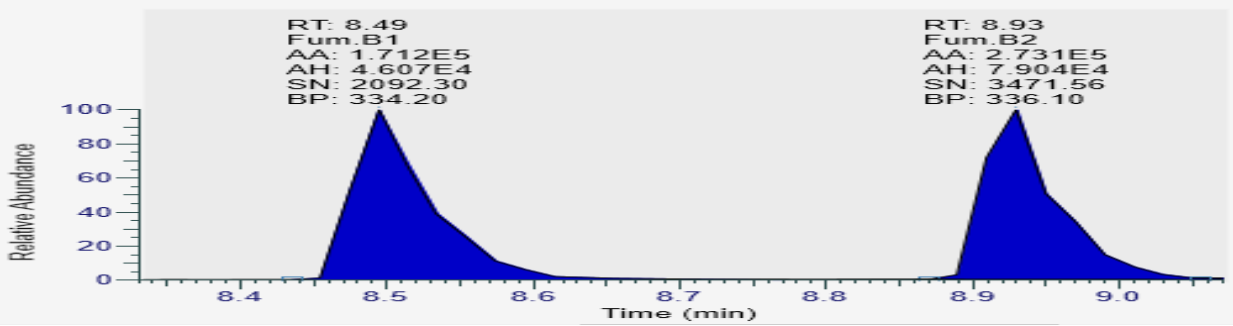

Figure 2:Extracted ion chromatogram of FB1 and FB2 by Eclips column (B).

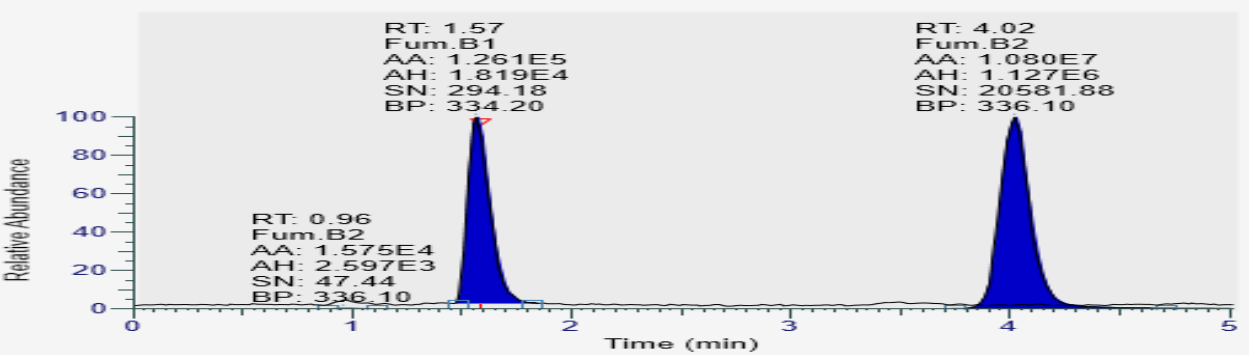

Figure 3:Extracted ion chromatogram of FB1 and FB2 by Poroshell column(C).

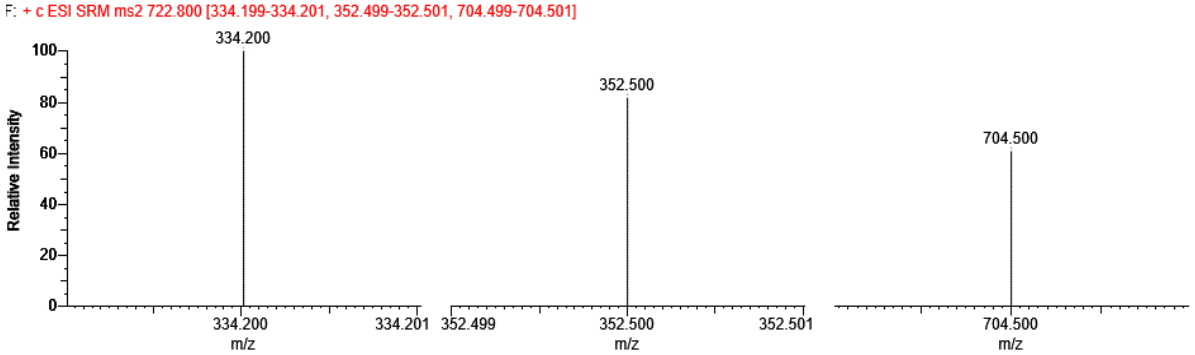

Figure 4:FB1 MRMs showing the quantifier ion and the two confirmatory qualifier ions.

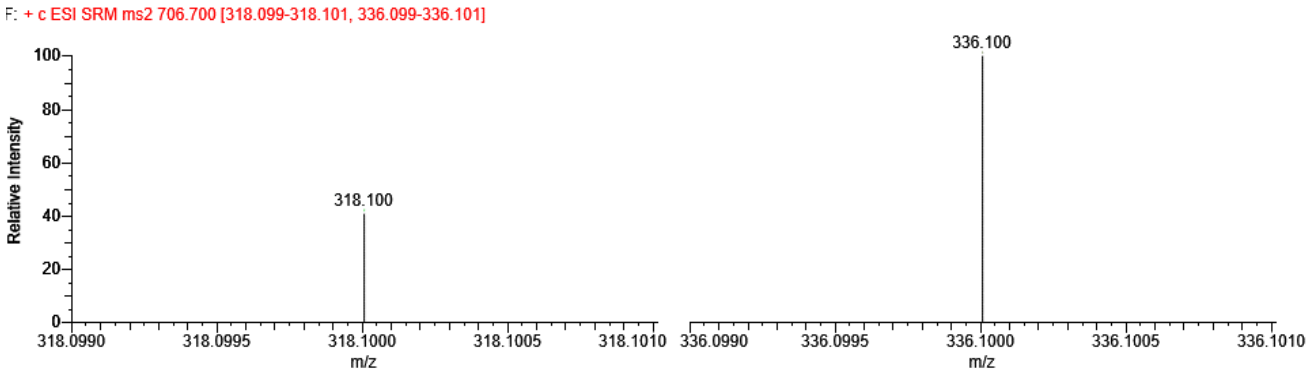

Figure 5:FB2 MRMs showing the quantifier ion and the confirmatory qualifier ion.

Table 2: MS/MS transitions and optimal operational conditions used for analysis**.**

Mycotoxin	Precursor ion (m/z)	Transition ion (m/z)	RT (min)	CE (V)
Fum.B1	722.8a	334.2	1.57	38
Fum.B1	722.8b	352.5	1.57	37
Fum.B1	722.8b	704.5	4.02	28
Fum.B2	706.7a	318.1	4.02	42
Fum.B2	706.7b	336.1	4.02	35

RT, retention time (min); CE, collision energy (volt); a product ion for quantifier peaks; b product ion for qualifier peaks.

3.2. Method validation

One purpose of this study was to confirm that analytical results were fit for the purpose. Method validation was tested following the Eurachem guidelines [39]. Method linearity, LOD, LOQ, percent recovery, repeatability, reproducibility, accuracy, and measurement of uncertainty have been measured.

3.2.1. LOQ and LOD

The sensitivity of the method has been determined with limits of quantitation (LOQ) and limit of detection (LOD). LOD has been valued according to Eurachem guidelines as 3 times SD, where s' is the standard deviation of the lowest level has been divided by the square root of the number of duplicates. LOD was 1.2, and 0.7 µg/Kg for FB1 and FB2 respectively. Also, LOQ has been estimated by using repeated spiked samples at about the expected lowest quantitation level and were 3.9 and 2.3 µg/Kg for FB1 and FB2 respectively.

The values obtained herein for the LOQ were lower than the values reported by Gazzotti et al., 2011 (10 µg/Kg for FB1 and FB) [40]; In Bryla et al., 2016 (12.5 µg/Kg for FB1and FB2) [41] in which evaporation and dissolution steps were used, as well as extract purification using SPE columns, which were not applied herein in the proposed method. Also, the LOQ was lower than the value reported by Andrade et al., 2017 (19 and 8 µg/Kg for FB1 and FB2, respectively) [26]; Hu et al., 2019 (28 µg/Kg for FB1 and 27 µg/Kg for FB2) [25]. While the LOQ was closed related to de Matos et al., 2021 which ranged from 1.3 to 6.59 µg/Kg for FB1 and from 0.60 to 4.60 µg/Kg for FB2 [34]. In Franco et al. 2018 LOQ was less than the present method for FB1 (2.8 µg/Kg) but highest in FB2 (2.5 µg/Kg) [33]. All of these methods are based on the Dilute-and-Shoot technique with using the lapelled internal stander in the injection on LC-MS/MS.

3.2.2. Instrument linearity

The multilevel calibration curve was plotted by using seven-point levels (2.5,25,50,250,500,750,1000) µg/Kg from intermediate solutions mixture of FB1 and FB2 (1mg/L). By conducting recovery tests at three different levels (20, 50, and 100 µg/Kg) linearity of the method was demonstrated by test results that have been directly proportional to the analyte concentration in the replicates for FB1 and FB2. The method was found to be linear from the 2.5 to 1000 µg/Kg with r =0.9999 for FB1 and FB2.

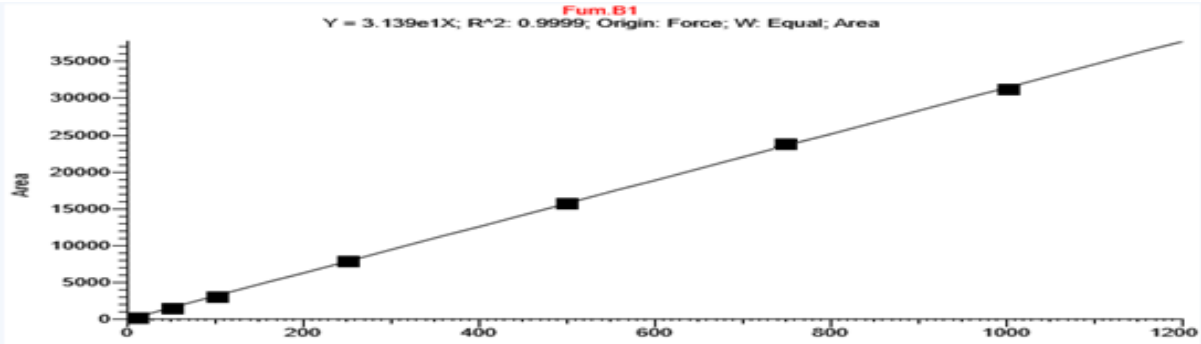

Figure 6: Calibration levels of FB1 ranged from 2.5 µg/Kg to 1000 µg/Kg.

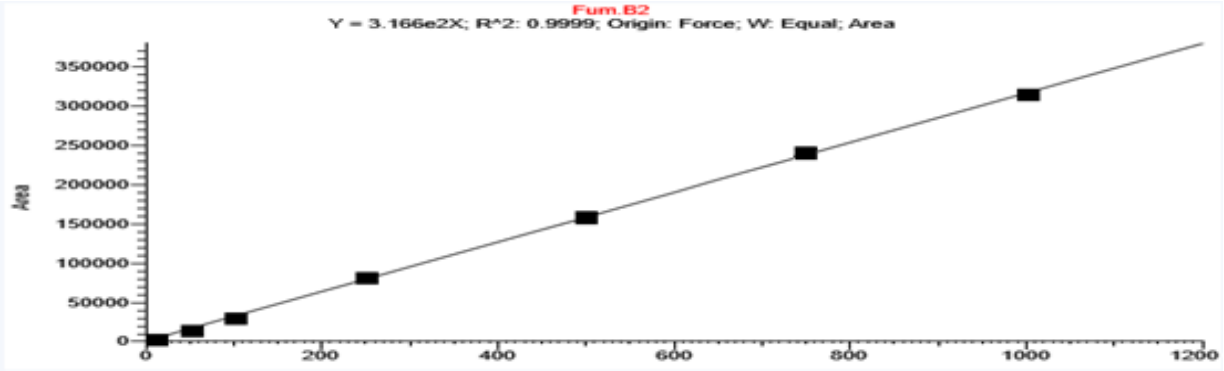

Figure 7: Calibration levels of FB1 ranged from 2.5 µg/Kg to 1000 µg/Kg.

3.2.3. Recovery test

The recovery test has been evaluated by performing three different levels of corn seed testing at 20, 50, and 100 µg/Kg. As shown in Table 3, the results ranged from 90.1% to 113.6 %, which corresponded to the European Commission regulation [42].

Table 3: Validation parameters: recovery, LOD, LOQ, and linearity of Fumonisin B1 and B2 in oilseeds

Mycotoxin		Spiking Level (µg/Kg)			LOD (µg/Kg)	LOQ (µg/Kg)
		20	50	100		
FB1	Recovery (%)	101.2	108.9	96.8	1.2	3.9
	± SD (%)	4.8	5.7	4.9		
	CV%	4.7	5.3	5.0		
FB2	Recovery (%)	104.6	105.1	100.1	0.7	2.3
	± SD (%)	2.8	1.4	1.2		
	CV%	2.7	1.3	1.2		

SD, standard deviation; CV, Coefficient of variation; LOD, limits of detection; LOQ, limits of quantitation.

3.2.4. Uncertainty

Measurement uncertainty sources have been estimated using precision (within-lab reproducibility) and bias data, according to Eurachem guidelines Table 4. When the combined and expanded uncertainty results were computed, the expanded uncertainty was found to be 25.2%. This low uncertainty can be justified by the method's high precision and robustness.

Table 4: Uncertainty measurement components of FBs in Corn seeds.

Description	**Relative Standard Uncertainty**
Standard Preparation (%)	0.7%
Bias (%)	2.4%
Sample Processing (%)	10.0%
Precision (%)	7.3%
Combined Uncertainty (%)	12.6%
Expanded Uncertainty (%)	25.2%

3.2.5. Method accuracy

The method recoveries of spiked samples were 90.1% to 117.9%. Also, the validity of this method has been determined by participating in Proficiency Tests organized by FAPAS. The result accepted Z-score should be between ±2. Table 5 displays the result of the FAPAS Proficiency Tests (PT). These findings provide more evidence for the method's performance in terms of high accuracy and robustness.

Table 5: Result of the FAPAS Proficiency Tests (PT).

PT 04418 Maize (2021)	FB1(µg/Kg)	FB2 (µg/Kg)	Total Fumonisin (µg/Kg)
Found	330	385.4	805.9
Assigned	420	414	720
Z-Score	1.4	-0.4	0.7

3.2.6. Method precision

Repeatability and reproducibility were applied to measure the precision of the obtained results. Six replicates from the same corn sample have been used to calculate the repeatability. The coefficient of Variation (CV) percent was 5.3% for FB1 and 1.3% for FB2. Reproducibility has been calculated at the intermediate precision using 20 different samples of corn as recommended by the Eurachem validation protocol (more than ten replicates). CV percent were 5.42% for FB1 and 4.10% for FB2.

3.2.7. Matrix effect

25 µl of standard solution with a concentration level of 1mg/L has been added to 475 µl of blank matrix extract to get matrix standard with the concentration of 50 µg/L, the dilution in the matrix will be only 5%.The matrix effect recovery in corn was more than 85% which corresponded to the European Commission regulation [42].

Table 6: Summary of the analytical method parameters used to analyze FB1 and FB2.

Method	Extraction Solvent	Assay time	Column	Run Time	LOQ
Gazzotti et al.2011[40].	Me OH :water (80:20)	>50Min	Waters XTerra MS C18 column (5 µm, 2.15 ×150 mm).	17 Min	FB1: 10 FB2: 10
Bryła et al. 2016 [41].	ACN : Me OH :Water (25:25:50)	>50Min	Kinetex PFP (100 mm ×2.1 mm, 2.6 mm) column.	55 Min	FB1:12.5 FB2:12.5
Andrade et al.2017[26].	ACN : Water :Acetic acid (80:20:0.1)	45 min	Gemini C18 analytical column (150 × 4.6 mm, 5µm).	12 Min	FB1:19 FB2:8
Franco et al. 2018 [33].	ACN : Water :Acetic acid (80:20:0.1)	95 Min	BEH C18 column (2.1 × 50 mm, 1.7 µm).	10 Min	FB1: 2.5 FB2: 2
Hu et al. 2019 [25].	ACN : Water :Acetic acid (70:29:1)	35 Min	Phenomenex Luna C18 column (150 mm × 2 mm, 3 µm)	15 Min	FB1: 28 FB2: 27
de Matos et al.2021[34].	ACN: Water: Formic acid (75:24:1)	20 min	BEH C18 column (100 mm × 2.1 mm, 1.7 µm).	4.5 Min	FB1: from 1.3 to 6.59 FB2: from 0.6 to 4.6
Present method	ACN: Water: Formic acid (79:20:1)	25 Min	(Poroshell 120 EC- C18 column (50 mm × 3 mm,2.7 µm)	5 Min	FB1: 3.9 FB2: 2.3

LOQ, limits of quantitation

3.3. Real samples

The test method has been used to quantify FB1 and FB2 in 300 samples taken from different seed retailers, fodder shops, and fields in various governorates of Egypt (i.e., Cairo, AL-Giza Al-Qalyubia, Al-Gharbia, Al-Bahira, and Al-Menoufia) in 2020 and 2021. All 120 samples of corn and white corn were contaminant by FB1 and FB2 .levels ranging in corn were from 9.7 to 8483.1µg/Kg (mean, 2136.4) µg/Kg for FB1 and from 1.8 to 1463.9µg/Kg (mean, 407.5µg/Kg) for FB2.while in white corn levels ranging was from 1.4 to 5557.5 µg/Kg (mean, 1140.9) µg/Kg for FB1 and from 0.6 to 987.7 µg/Kg (mean, 213.2 µg/Kg) for FB2. 28% of corn samples and 20% of white corn samples exceeded the maximum permissible limits of the Sum of FB1 and FB2 which was 4 mg/Kg according to the regulation of Eu. 72% of corn samples and 80 % of white corn Samples were less than 4 mg/Kg. The highest infection from FB1 in corn was 8483 µg/Kg and it was in the Al-Gharbia governorate, while in white corn FB1 was 5557.5µg/Kg and it was in the Al-Bahira governorate. Also, the highest infection from FB2 in Corn was 2084.3 µg/Kg and it was in the Al-Giza governorate also. While in white corn FB2 was 987.7µg/Kg and it was in the Al-Gharbia governorate. The highest concentration of the sum of FB1 and FB2 was in corn 10034.9 µg/Kg and it was in Al-Gharbia governorate while in white corn was 5821.3 µg/Kg and it was in Al-Bahira governorate.

Table 7: Contamination range and the occurrence of FB1 and FB2 in some seed samples collected from some Egyptian governorates (data in µg/kg).

Governorate	Seeds	FB1(µg/Kg)	FB2(µg/Kg)	Date of detection
Cairo	White corn	<LOQ - 4327.2	<LOQ - 825.1	August 2020
	Corn	13.1 – 8072.1	3.1 - 1424.7	
	Soybean	n.d. - 14.3	n.d.	
	Sesame	n.d.	n.d.	
	Sunflower	n.d.	n.d.	
Al-Giza	White corn	<LOQ - 4442.3	<LOQ - 965.1	October 2020
	Corn	37.2 – 8483.1	9.6 – 2084.3	
	Soybean	n.d. - 157.3	n.d. - 46.6	
	Sesame	n.d.	n.d.	
	Sunflower	n.d.	n.d.	
Al-Menoufia	White corn	<LOQ - 4828.9	<LOQ -542.6	September 2020
	Corn	18.2 - 6881.8	3.5 - 1435.9	
	Soybean	n.d. – 66.3	n.d. – 13.6	
	Sesame	n.d.	n.d.	
	Sunflower	n.d.	n.d.	
Al-Qalyubia	White corn	<LOQ - 4962.2	<LOQ - 882.4	August 2021
	Corn	98.7 - 5397.4	14.5 - 1071.5	
	Soybean	n.d. – 276.3	n.d. – 51.6	
	Sesame	n.d.	n.d.	
	Sunflower	n.d.	n.d.	
Al-Beheira	White corn	<LOQ - 5557.5	<LOQ - 928.8	September 2021
	Corn	9.7 - 7229.2	<LOQ - 1312.7	
	Soybean	n.d – 47.2	n.d. – 8.6	
	Sesame	n.d.	n.d.	
	Sunflower	n.d.	n.d.	
Al-Gharbia	White corn	4.6 - 3492.3	<LOQ - 987.7	October 2021
	Corn	39.7 – 8571	6.6 - 1463.9	
	Soybean	n.d. – 41.79	n.d. – 11.2	
	Sesame	n.d.	n.d.	
	Sunflower	n.d.	n.d.	
Total range	White corn	<LOQ - 5557.5	<LOQ - 987.7	2020-2021
	Corn	9.7 – 8571	<LOQ – 2084.3	
	Soybean	n.d. - 276.3	n.d. – 51.6	
	Sesame	n.d.	n.d.	
	Sunflower	n.d.	n.d.	

n.d., not detected; LOQ, limits of quantitation. = 3.9 µg/Kg for FB1 and 2.3 µg/Kg for FB2.

In soybean, 77% of 60 samples were free of FB1 and FB2.while 23 % of samples were contaminant by FB1 with levels ranging from 14.3 to 276.3 µg/Kg (mean, 12.2) µg/Kg .20% of samples were contaminant by FB2 with levels ranging from 8.6 to 51.6 µg/Kg (mean, 2.7) µg/Kg. The highest infection from FB1 was 276.3 µg/Kg and it was in the Qalyubia governorate. While FB2 was 51.6 and it was in the Qalyubia governorate. Also, the highest concentration of the sum of FB1 and FB2 was 321.9 µg/Kg and it was in the Qalyubia governorate. In sesame and sunflower seeds, all 120 samples were free of FB1 and FB2.

These findings have been compared in corn to those from other countries The results were close to Thailand, where FB1 and FB2 were detected in 89% and 67% of the samples at concentrations going from 63 to 18800 g/Kg (mean, 1790 g/Kg) and 50 to 1400 g/Kg (mean, 251 g/Kg), respectively[35]. Also, In Iran, The research was divided into two places Mazandaran and Isfahan. All the samples from Mazandaran had been shown high levels of contamination of fumonisin with FB1 levels between 1270 and 3980 µg/Kg, and FB2 levels between 190 and 1175 µg/Kg, While the samples from Isfahan had been shown lower contamination levels of FBs. Eight of the eight

samples had detectable FB1 (10-590 g/Kg), and two of the eight samples had detectable FB2 (50-75 g/Kg) [36]. In China, Except for one sample, all were 99.6% positive for FB1 at levels going from 3 to 71,121 g/Kg, with average and median levels of 6,662 and 1,569 g/Kg, respectively. 43.6 percent of samples had FB1 concentrations less than 1,000 g/Kg, while 25.2 percent had concentrations greater than 5,000 g/Kg [37].

Also, this investigation reveals that compared to the previous study in Egypt in 2009 [38], the level of contamination by Fumonisin in Egypt has been reduced during the past decade. This improvement in Fumonisin levels could be attributed to the improvement of the food control system of Egypt and the continuous monitoring of local markets in Egypt.

4. Conclusion

An LC-MS/MS analytical method for determining FB1 and FB2 in oilseeds has been optimized and validated in this study. The fumonisin determination sample treatment method was direct, relying on solvent extraction with fast shaking without any need for clean-up steps. The method has been validated to characterize the analytical parameters and confirm that the test method under consideration had performance capabilities that were consistent with the application's requirements according to Eurachem guidelines. Also, The Finish Accreditation Service (FINAS) has granted this method international accreditation for meeting the requirements of the ISO/IEC 17025:2017 standard. Furthermore, the proposed method applied for analyze 300 oilseeds samples, and the results indicated that more than 27% of the corn and 20% of white corn samples had been exceeded the regulation of the EU. So, It is necessary to take action and set relevant preventive measures by conducting periodic analyzes of incoming corn shipments before entering the Egyptian market.

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