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Multiclass mycotoxin analysis in edible oils using a simple solvent extraction method and liquid chromatography with tandem mass spectrometry

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ABSTRACT

A simple and rapid method for the simultaneous determination of 11 mycotoxins – aflatoxins B_1, B_2, G_1 and G_2; fumonisins B_1, B_2 and B_3; ochratoxin A; zearalenone; deoxynivalenol; and T-2 toxin – in edible oils was established using liquid chromatography tandem mass spectrometry (LC-MS/MS). In this study, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), QuEChERS with dispersive liquid–liquid microextraction, and solvent extraction were examined for sample preparation. Among these methods, solvent extraction with a mixture of formic acid/acetonitrile (5/95, v/v) successfully extracted all target mycotoxins. Subsequently, a defatting process using n-hexane was employed to remove the fats present in the edible oil samples. Mass spectrometry was carried out using electrospray ionisation in polarity switching mode with multiple reaction monitoring. The developed LC-MS/MS method was validated by assessing the specificity, linearity, recovery, limit of quantification (LOQ), accuracy and precision with reference to Commission Regulation (EC) 401/2006. Mycotoxin recoveries of 51.6–82.8% were achieved in addition to LOQs ranging from 0.025 ng/g to 1 ng/g. The edible oils proved to be relatively uncomplicated matrices and the developed method was applied to 9 edible oil samples, including soybean oil, corn oil and rice bran oil, to evaluate potential mycotoxin contamination. The levels of detection were significantly lower than the international regulatory standards. Therefore, we expect that our developed method, based on simple, two-step sample preparation process, will be suitable for the large-scale screening of mycotoxin contamination in edible oils.

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Simultaneous analysis; multiclass mycotoxin; edible oil; LC-MS/MS

Introduction

Mycotoxins are secondary metabolites mainly produced by the *Aspergillus, Penicillium*, and *Fusarium* species, and are toxic to both animals and humans. Although there are tens of thousands of fungi in nature, only about 400 mycotoxins have been discovered to date. In general, fungi are susceptible to heat and can be destroyed by heating to 50–60°C. However, the majority of mycotoxins are heat-stable and cannot be completely removed by such methods (Zinedine and Mañes 2009). Although mycotoxins do not cause immediate physical abnormalities at typical exposure levels, their long-term ingestion can cause chronic illnesses in the liver and kidneys. (Peraica et al. 1999). A number of mycotoxins are also carcinogenic and genotoxic, and can cause serious health and hygiene problems (Zinedine and Mañes 2009; World Health Organization 2016). Mycotoxin contamination usually occurs in cereals such as rice, corn, wheat, barleyand soybean. In addition, processed foods based on these materials have a high risk of contamination. Mycotoxins can be produced throughout the processes of food production, from drying and storage of the raw commodities, and during the manufacturing and storage of foods and their distribution. In addition, as international trade volumes increase, the long-term storage of foods and other raw materials is increasing. Therefore, it is necessary to investigate the actual conditions of mycotoxin contamination.

To date, various reports have discussed mycotoxin contamination in cereals and nuts, which are the raw materials of edible oils (Shephard et al. 2000; Schollenberger et al. 2005; Briones-Reyes et al. 2007; Lutfullah and Hussain 2012; Wu et al. 2016).

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However, there has been few studies on mycotoxin contamination of edible oils. In Korea, regulations regarding the acceptable limits of the major mycotoxins have been established for cereals and nuts; however, no such regulations have been introduced for vegetable oils from corn and soybeans (Korea Food Standards Codex 2016). The European Union (EU) set the maximum allowable level of zearalenone in refined maize oil at 200 μg/kg in 2006, and changed this value to 400 μg/kg in 2007 (Commission 2007; Siegel et al. 2010). China also sets limits for aflatoxin content in fat and its products as 10–20 μg/kg (Chinese National Standard 2011).

As mycotoxins are present at particularly low levels (μg/kg) in foodstuffs, methods for their qualitative and quantitative analysis must be both sensitive and selective. For selective determination, effective separation is essential and can be achieved using chromatographic techniques such as gas chromatography (GC) and liquid chromatography (LC) (Frenich et al. 2011). Previously, HPLC-UV/Vis, HPLC-FLD and HPLC-MS were used for mycotoxin analysis in foods (Arroyo-Manzanares et al. 2013a). However, LC-MS/MS enables more efficient analysis and has many advantages over commonly used methods (Sharmili et al. 2016), as it can be used to detect compounds selectively and accurately using their mass-to-charge ratios (m/z). Structural information can also be obtained from the fragmentation patterns.

To date, mycotoxin analysis in edible oils has received little attention compared to the analysis of raw materials. While considering the fatty properties of edible oils, various sample preparation methods have been studied, including matrix solid phase dispersion (MSPD) (Cavaliere et al. 2007), liquid–liquid extraction (LLE) (Idris et al. 2010), immunoaffinity column (IAC) (Bao et al. 2012), IAC combined with dispersive liquid–liquid microextraction (DLLME) (Afzali et al. 2012), automated solid phase extraction (SPE) (Drzymala et al. 2015) and gel permeation chromatography (GPC) (Qian et al. 2015). In addition, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method is a widely used approach for mycotoxin determination in other types of foods. This method is of particular interest as it does not require substantial technical expertise and uses small quantities of organic solvents compared to other methods (Pereira et al. 2014). Since this method consists of two steps, namely salting-out extraction and dispersive SPE (dSPE), it is possible to carry out the simple extraction of target components and remove unwanted components from complicated samples. Notably, this method has been applied to mycotoxin analysis in edible oils (Sharmili et al. 2016) and herbal medicines, the latter of which are relatively complex matrices (Arroyo-Manzanares et al. 2013a).

The purpose of this study is therefore to establish a simple and rapid methodology based on LC-MS/MS for the simultaneous determination of mycotoxins in edible oils. The mycotoxins regulated by the Korea Food Standards Codex are total aflatoxins (sum of B_1, B_2, G_1 and G_2), aflatoxin M_1, fumonisins (sum of B_1 and B_2), ochratoxin A, deoxynivalenol, zearalenone and patulin. In this study, aflatoxin M_1 and patulin were excluded as they are specific for milk and infant formula, and apple juice. Including fumonisin B_3 and T-2 toxin, a total of 11 mycotoxins were examined as target components. The developed method was applied to the analysis of nine real edible oil samples.

Materials and methods

Reagents and materials

Acetonitrile, methanol, water and chloroform were purchased as HPLC- or LC/MS-grade high-purity solvents from Fisher Scientific Korea (Seoul, Korea). n-Hexane was purchased from Wako Pure Chemical Industries (Tokyo, Japan). Formic acid, acetic acid, ammonium acetate and ammonium formate were HPLC or LC/MS grade and were purchased from Sigma-Aldrich (St Louis, MO, U.S.A.).

Anhydrous magnesium sulfate and sodium chloride were purchased from Daejung Chemicals & Metals (Siheung, Korea). Sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate were purchased from Sigma-Aldrich. The d-SPE sorbent, end-capped C18 bulk packing, was obtained from Sigma-Aldrich. Syringe filters (0.2 μm) with PTFE membranes were purchased from Whatman (Maidstone, UK).

Preparation of standard solutions

An aflatoxins standard was purchased from Trilogy Analytical Laboratory Inc. (Washington, MO, U.S.A.) as a mixed solution dissolved in acetonitrile, in which concentrations of B_1, B_2, G_1 and G_2 were 2, 0.5, 2 and 0.5 μg/mL, respectively. Fumonisin B_1 and B_2 standard

was purchased from Sigma-Aldrich as a mixed solution dissolved in acetonitrile each at a concentration of 50 μg/mL. Fumonisin B_3 standard was purchased as a 50 μg/mL solution dissolved in water/acetonitrile (50/50, v/v) from Wako Pure Chemical Industries. Ochratoxin A and T-2 toxin standards were purchased from Sigma-Aldrich as 10 and 100 μg/mL solutions dissolved in acetonitrile, respectively. Deoxynivalenol standard was purchased from Sigma-Aldrich and the stock solution was prepared by dissolving the standard in methanol at a concentration of 200 μg/mL. Zearalenone standard was provided by the Ministry of Food and Drug Safety as a 100 μg/mL solution dissolved in acetonitrile. Ochratoxin A-d5 standard was purchased from Sigma-Aldrich and used as an internal standard.

The aflatoxin mixture standard was stored at 4°C and others were stored at −20°C. Working solutions were freshly prepared by diluting the standard solutions with acetonitrile.

Samples

Nine edible oil samples were purchased from Hanaro Mart (Seoul, Korea), including three soybean oil samples, three corn oil samples and three rice bran oil samples. All samples were stored at room temperature away from direct sunlight.

Instruments and equipment

Liquid chromatography was performed on an Agilent 1290 Infinity system from Agilent Technologies (Santa Clara, CA, U.S.A.) and mass spectrometry was performed on an Agilent 6490 triple quadrupole from Agilent Technologies. A Phenomenex Kinetex C18 column (100 mm × 2.1 mm, 1.7 μm) equipped with a SecurityGuard ULTRA guard column was used.

A vortex mixer (Vortex genie-2, Scientific Industries, Bohemia, NY, U.S.A.), centrifuge (MF-550, Hanil Science Industrial, Incheon, Korea), shaker (Green SSeriker, Vision Scientific, Daejeon, Korea) and ultrasonic cleaner (Daihan Scientific, Seoul, Korea) were also used in this study.

Data analysis was performed using Agilent LC/MS Data Acquisition for 6400 Series Triple Quadrupole (ver.B.07.00), Agilent MassHunter Qualitative Analysis (ver.B.06.00) and Agilent MassHunter Quantitative Analysis (ver.B.06.00).

Optimised procedures

QuEChERS

The sample (2 g) was weighed into a 50 mL conical tube. For the extraction step, 7 mL of water and 10 mL of acetonitrile/formic acid (95/5, v/v) were added and vortexed for 3 min. Then, the extraction salts (4 g of $MgSO_4$, 1 g of NaCl, 1 g of sodium citrate tribasic dihydrate and 0.5 g of sodium citrate dibasic sesquihydrate) were added, shaken for 10 min, and centrifuged at 2,200 g for 7 min. For the dSPE purification process, the supernatant (7 mL; acetonitrile layer) was transferred into another tube and the dSPE sorbents (750 mg of $MgSO_4$ and 250 mg of C18) were added. The mixture was vortexed for 10 min and centrifuged at 2200 g for 7 min. Next, the supernatant (2.5 mL; acetonitrile layer) was transferred into a round bottomed tube, evaporated under a gentle stream of nitrogen at 45°C, and reconstituted with 500 μL of 0.1% formic acid (water)/acetonitrile (50/50, v/v). The final extract was filtered with a 0.2 μm syringe filter and injected into the LC-MS/MS instrument.

QuEChERS × DLLME

The QuChERS × DLLME process was as described above for the QuEChERS process until the point of reconstitution, for which 1 mL of water/methanol (50/50, v/v) was employed. Then, 4 mL of water, 0.21 g of NaCl, 0.6 mL of chloroform and 0.9 mL of acetonitrile were added, and the mixture was shaken and centrifuged at 2200 g for 7 min. The lower layer (chloroform layer) was transferred into another test tube, evaporated under a gentle stream of nitrogen at 45°C, and reconstituted with 500 μL of 0.1% formic acid (water)/acetonitrile (50/50, v/v). The final extract was filtered with a 0.2 μm syringe filter and injected into the LC-MS/MS instrument.

Solvent extraction

The sample (2 g) was weighed into a 50 mL conical tube and 10 mL of acetonitrile/formic acid (95/5, v/v) were added. The mixture was vortexed for 30 min and centrifuged at 2200 g for 7 min. The supernatant (5 mL; acetonitrile layer) was transferred into another tube and 5 mL of n-hexane (acetonitrile saturated) were added.

The tube was vortexed for 10 min and centrifuged at 2200 g for 5 min. The lower layer (2.5 mL; acetonitrile layer) was transferred into a round-bottomed tube, evaporated under a gentle stream of nitrogen gas at 45°C, and reconstituted with 500 µL of an internal standard solution (ochratoxin A-d_5 10 µg/mL in water/methanol (50/50, v/v)). The final extract was filtered with a 0.2 µm syringe filter and injected into the LC-MS/MS instrument (see Figure 1).

LC-MS/MS conditions

Chromatographic separations were achieved on an ODS column (Kinetex C18 column) using 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B) as the mobile phases. The gradient profile is outlined in Table 1. The flow rate was 0.2 mL/min, the column temperature was 30°C and the injection volume was 5 µL.

Triple quadrupole tandem mass spectrometry (MS/MS) was used for the detection of each component separated by UHPLC. MS/MS analysis was performed with electrospray ionisation (ESI) in polarity switching mode, which was suitable for the simultaneous analysis of positive and negative ions. The ESI conditions were as follows: drying gas flow rate was 11 L/min; drying gas temperature was 240°C; nebuliser was 40 psi; sheath gas flow rate was 10 L/min; sheath gas temperature was 400°C; capillary voltage was 4000 V for positive and 3500 V for negative; nozzle voltage was 500 V for positive and 2000 V for negative.

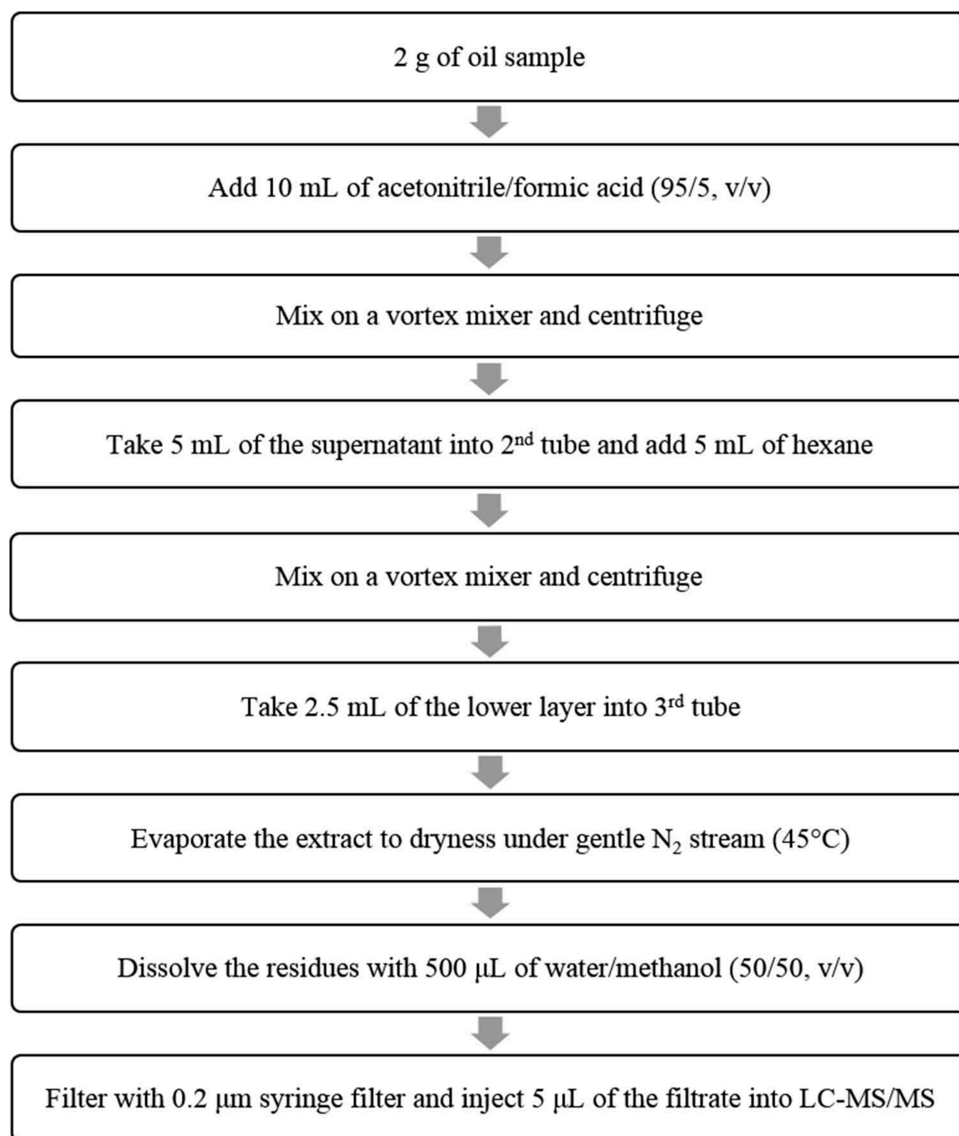

Figure 1. Flow diagram for the proposed solvent extraction method.

Table 1. Optimised gradient profiles of the chromatographic separations.

Time (min)	A (%)	B (%)
0	90	10
1	90	10
2	50	50
6	50	50
7	40	60
15	35	65
16	10	90
22	10	90
23	90	10
28	90	10

Multiple reaction monitoring (MRM) was used to improve the selectivity and sensitivity of detection. The collision energy was optimised for each compound to obtain the highest fragment ion response. The fragment ion with the highest intensity was selected as the quantification ion and the second and third most intense ions were used as confirmation ions. The optimised MRM parameters for each compound are outlined in Table 2.

Method validation

The developed LC-MS/MS method was validated by assessing the specificity, linearity, recovery, limit of quantification (LOQ), accuracy and precision, with reference to commission regulation (EC) No. 401/2006 (Commission 2006).

To assess the specificity, the lack of interfering matrix peaks was confirmed in the chromatograms of three blank samples. The linearity was evaluated with standard-spiked samples. The concentration range was adjusted to 5 or 6 points depending on the components, and a plot of the peak area ratio (peak area of analyte/peak area of internal standard) for each compound was prepared. The correlation coefficient (R^2) was evaluated. The recovery was determined by comparing the standard-spiked samples and control samples, which were matrix-matched standards prepared by adding the standard solution to the extract obtained by preparation of the blank samples. The recoveries required for the analytical method depended on each individual mycotoxin concentration. The various recoveries were as follows: aflatoxins > 50–80%; fumonisins and T-2 toxin > 60%; ochratoxin A > 50–70%; and zearalenone and deoxynivalenol > 60–70%. The accuracy and precision were evaluated from the recovery data. The accuracy was assessed using the three-time analysis of the recovery, and the precision was confirmed by the relative standard deviations (RSDs) of the recovery values. RSD values <20–40% were required depending on the mycotoxin type and concentration. The LOQs were determined as the concentrations that exhibited a signal-to-noise ratio (S/N ratio) of at least 10 in the LC-MS/MS chromatograms of the standard-spiked samples. In detail, blank samples were spiked with standard solutions and underwent the all analytical procedures and the lowest concentration with a S/N ratio of at least 10 was determined as LOQ for each component.

Table 2. Optimised MRM parameters for each compound.

Mycotoxins	Precursor ion (m/z)	Product ions (m/z)	
		Quant. ion (CE[a])	Qual. ions (CE[a])
$AFTB_1$	313.1 $[M + H]^+$	240.9 (41)	268.9 (34), 285.0 (22)
$AFTB_2$	314.9 $[M + H]^+$	258.9 (31)	286.9 (27), 242.9 (46)
$AFTG_1$	329.0 $[M + H]^+$	242.8 (28)	299.8 (45), 311.0 (21)
$AFTG_2$	331.0 $[M + H]^+$	245.0 (32)	285.0 (30), 188.9 (46)
OTA	402.1 $[M - H]^-$	358.1 (20)	210.9 (30), 166.9 (40)
ZEA	317.1 $[M - H]^-$	174.9 (26)	273.0 (20), 131.0 (32)
DON	297.0 $[M + H]^+$	249.1 (8)	203.1 (13), 231.0 (9)
$FUMB_1$	722.3 $[M + H]^+$	352.2 (42)	334.0 (46), 316.0 (44)
$FUMB_2$	706.3 $[M + H]^+$	336.2 (40)	318.2 (46), 140.9 (56)
$FUMB_3$	706.3 $[M + H]^+$	336.2 (40)	530.2 (33), 512.3 (34)
T-2	484.1 $[M + NH_4]^+$	214.9 (18)	305.0 (10), 185.1 (23)
OTA-d_5 (IS)	407.1 $[M - H]^-$	363.0 (20)	167.1 (44), 210.9 (29)

[a]CE: collision energy.

Monitoring of commercial samples

To monitor the levels of mycotoxin contamination in commercial samples, quantitative analysis was performed. Calibration curves were prepared using the samples spiked with standard solutions prior to extraction, and the appropriate calibration curves were prepared for the soybean oil, corn oil and rice bran oil samples.

Results and discussion

Optimisation of LC-MS/MS conditions

Ultra-high performance liquid chromatography (UHPLC) using sub-2 µm particles was used to detect multiple components in a single analysis within a short period of time, and to achieve a superior separation efficiency compared to conventional liquid chromatography.

In order to obtain optimal sensitivity, the effects of various additives (formic acid, acetic acid, ammonium formate and ammonium acetate) and organic solvents (methanol and acetonitrile) were compared.

There were differences in the sensitivities of the MS/MS detection for each component, and the sensitivities towards fumonisins and T-2 toxin were relatively lower than those of other components. Thus, the mobile phase conditions were optimised by prioritising the sensitivities for fumonisins and T-2 toxin. According to previous reports, mobile phases containing formic acid led to increased sensitivities towards fumonisins (Zachariasova et al. 2010). In addition, a combination of formic acid and ammonium formate produced the optimal sensitivity towards T-2 toxin, which was detected as its ammonium adduct. However, these conditions resulted in greater decreases in the sensitivities towards fumonisins than the corresponding increase in the sensitivity towards T-2 toxin. Thus, 0.1% formic acid in water (v/v) and 0.1% formic acid in methanol (v/v) were selected as the mobile phases, and the gradient profile was optimised to allow detection of all components within 15 minutes (see Table 1).

Figure 2 shows the chromatograms of the standard solutions obtained under the optimised LC-MS/MS conditions. The retention times were 3.6 min for deoxynivalenol, 4.9 min for aflatoxin G_2, 5.2 min for aflatoxin G_1, 5.7 min for aflatoxin B_2, 6.2 min for aflatoxin B_1, 9.4 min for fumonisin B_1, 10.4 min for T-2 toxin, 11.3 min for fumonisin B_2, 12.5 min for zearalenone, 13.2 min for ochratoxin A and 13.5 min for fumonisin B_3.

Optimisation of sample preparation

QuEChERS

Sample preparation was carried out based on the QuEChERS method, which is widely used for the analysis of mycotoxins in food. A modified procedure based on previously reported methods was utilised (Polgár et al. 2012; Arroyo-Manzanares et al. 2013a; Koesukwiwat et al. 2014). As acidic mycotoxins such as fumonisins and ochratoxin A exhibit good recoveries when acidic solvents are employed for the extraction (Zachariasova et al. 2010; Koesukwiwat et al. 2014), we used formic acid, which is a common acidic modifier. In regard to dSPE sorbents, C18, primary secondary amine (PSA) and graphite are typically used in QuEChERS. However, PSA and graphite have been

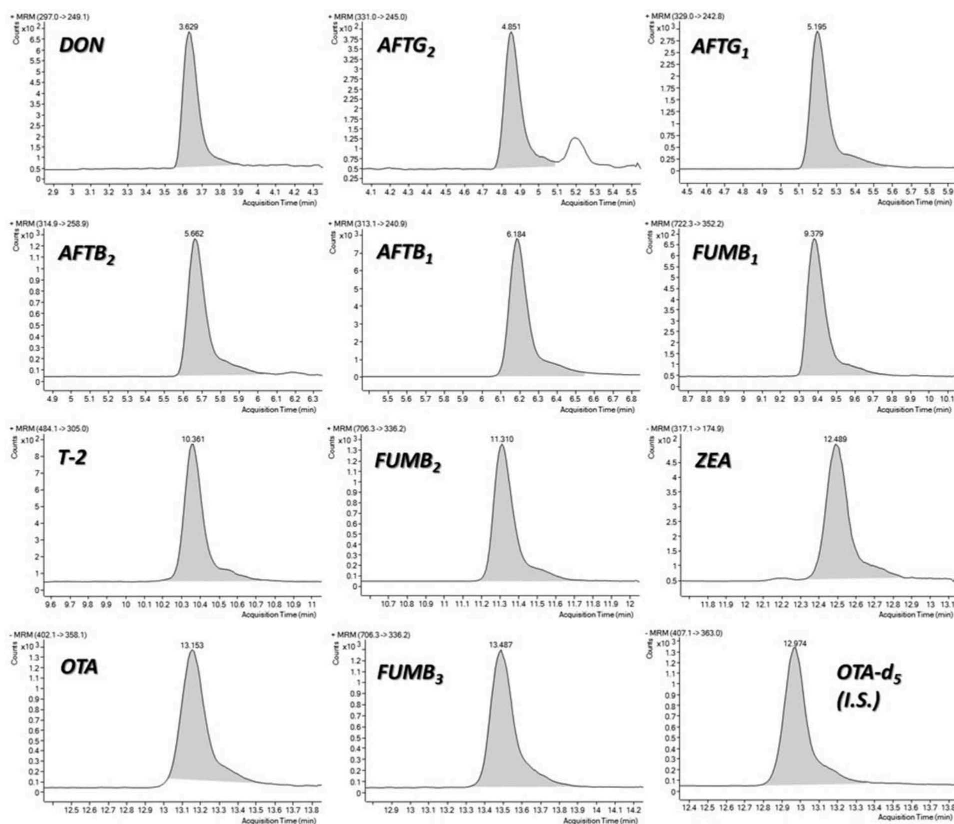

Figure 2. Extracted ion chromatograms of the quantification ions for 11 target mycotoxins and the internal standard (DON: deoxynivalenol; AFT: aflatoxin; FUM: fumonisin; ZEA: zearalenone; OTA: ochratoxin A).

reported to absorb some mycotoxins (Liu et al. 2014). We confirmed that fumonisins exhibited extremely low recoveries when PSA was used as the dSPE sorbent (data not shown). Therefore, we excluded other sorbents and used C18 as the dSPE sorbent for the purpose of this study. Under these conditions, the recoveries of aflatoxin B_1 and G_1 were remarkably low, while the remaining mycotoxins showed good recoveries of 65% or more.

In the QuEChERS method, acetonitrile-based extraction is typically used owing to the facile separation from water. In order to modify the effectiveness of extraction, methanol-based extraction was attempted. However, in the case of the methanol-based extraction, it seemed that greater quantities of oil components dissolved in the extract, and it was not easy to evaporate under nitrogen even after defatting with n-hexane. Therefore, we employed an acetonitrile-based solvent for the extraction process.

To determine the cause of the decreased recoveries, the results were evaluated by sequentially excluding the dSPE step and water addition during extraction (Figure 3(a)). The recoveries of aflatoxin B_1 and G_1 were steadily improved with the exclusion of the dSPE and water addition. However, without water addition, recoveries of fumonisins were decreased to less than half of the previous results. Recoveries other than for aflatoxin B_1, G_1 and fumonisins were little affected by water addition.

To determine the effects of excess salts in the extraction step without the addition of water, various combinations of extraction salts were utilised: (a) 4 g of $MgSO_4$, 1 g of NaCl, 1 g of sodium citrate tribasic dihydrate, 0.5 g of sodium citrate dibasic sesquihydrate; (b) 4 g of $MgSO_4$, 1 g of NaCl; (c) 1 g of NaCl; (d) no salt (Figure 3(b)). The recoveries of each component varied depending on the salt combinations. This change was more pronounced

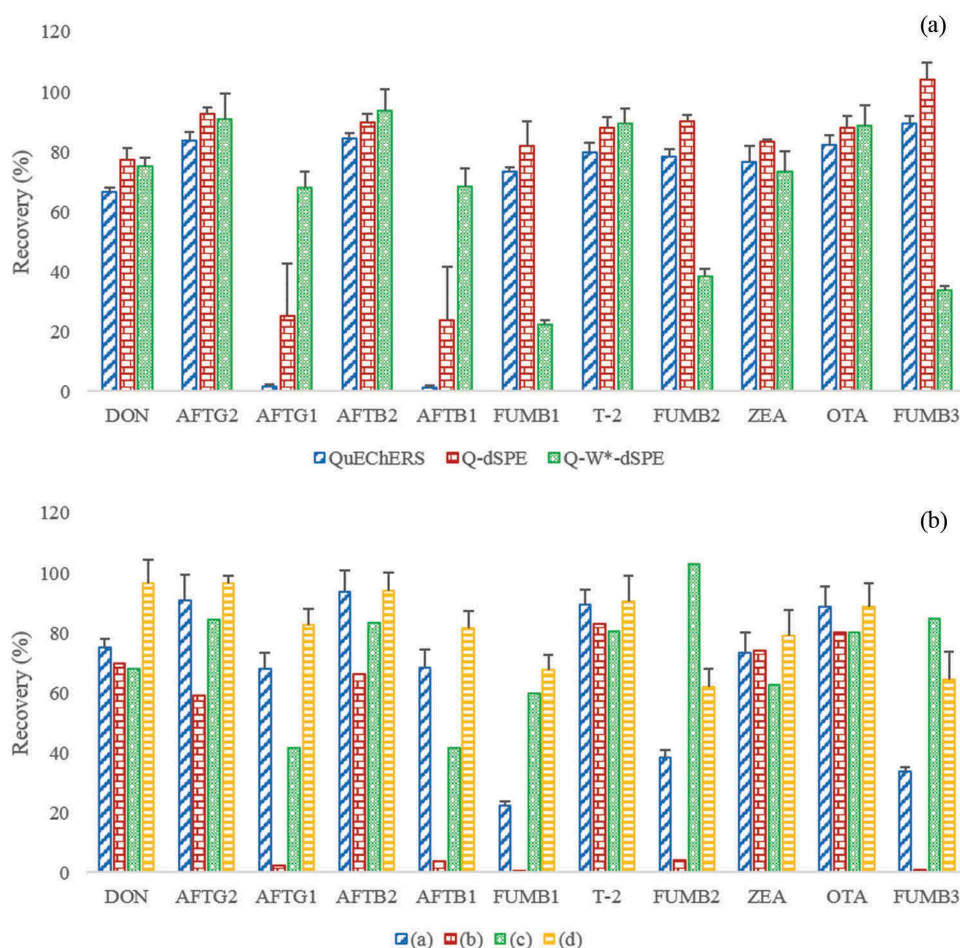

Figure 3. Comparison of recoveries with different variables: (a) dSPE purification process and water addition (* water addition during extraction) (n = 3); (b) various extraction salts ((a) 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate tribasic dihydrate, 0.5 g sodium citrate dibasic sesquihydrate; (b) 4 g $MgSO_4$, 1 g NaCl; (c) 1 g NaCl; (d) no salt) (n = 3 for (a) and (d), n = 1 for (b) and (c)).

with aflatoxin B_1, G_1 and fumonisins. However, the best result was obtained without extraction salts.

QuEChERS × DLLME

In the analysis of mycotoxins present in herbal medicines, DLLME after QuEChERS was reported to reduce matrix effects (MEs) and interfering peaks (Arroyo-Manzanares et al. 2013a). With this in mind, we evaluated the addition of DLLME to our procedure. A modified version of a previously reported (Arroyo-Manzanares et al. 2013a) was carried out. Unfortunately, the results were disappointing. As reported previously, deoxynivalenol and fumonisins exhibited extremely low recoveries. The recoveries of other mycotoxins were not improved, and even decreased by about 5% compared to the results of QuEChERS. In complex matrices such as herbal medicines, reducing the influence of MEs might be important for sensitive detection. However, edible oils are not complex matrices, as described in 'Matrix effects' section, and it seemed that the decrease in recoveries due to the additional sample preparation steps more than offsets the benefits of the additional clean up.

Solvent extraction

Based on the QuEChERS results, we expected that simple solvent extraction was suitable for extraction of mycotoxins from edible oils. Considering the fatty properties of edible oils, a defatting process after an extraction step was added. First, a freezing-out method was tested. The extract was stored at −20°C for 2 hours, but the oil layer hardly solidified. As an alternative, the procedure using n-hexane was employed; this resulted in slightly reduced recoveries, but it would reduce the contamination of ionisation source and aid in the durability of the instrument. After the defatting process was established, the optimisation of the extraction step was performed. To obtain the maximum recoveries, various acid contents in the extraction solvent were tried (see Figure 4). Formic acid was required to enhance recoveries of the acidic mycotoxins such as fumonisins and ochratoxin A, but the recovery of deoxynivalenol decreased as the acid content was increased. When the acid content was increased to 10%, the recoveries of all mycotoxins decreased. Overall, the best result was obtained with formic acid/acetonitrile (5/95, v/v). Next, to optimise the extraction and defatting steps, the reconstitution procedure was optimised. When the solvent containing acid was used to reconstitute, the peak corresponding to deoxynivalenol exhibited shouldering (see Figure 5). Deoxynivalenol was reported to be slightly unstable under acidic conditions (Mishra et al. 2014). For this reason, it seemed that the pH of the reconstitution solvent had an effect on the degradation of deoxynivalenol. In order to improve the peak shape, the reconstitution solvent was changed to water/methanol (50/50, v/v). The optimised procedure was described in the 'Procedures' section and Figure 1. The utility of the developed method was confirmed by determining the recoveries of mycotoxins from three kinds of edible oils (soybean oil, corn oil, and rice bran oil); the results are shown in Figure 6. The results of corn oil and rice bran oil were similar to that of soybean oil. Moreover, the recoveries of fumonisins were much higher from corn oil.

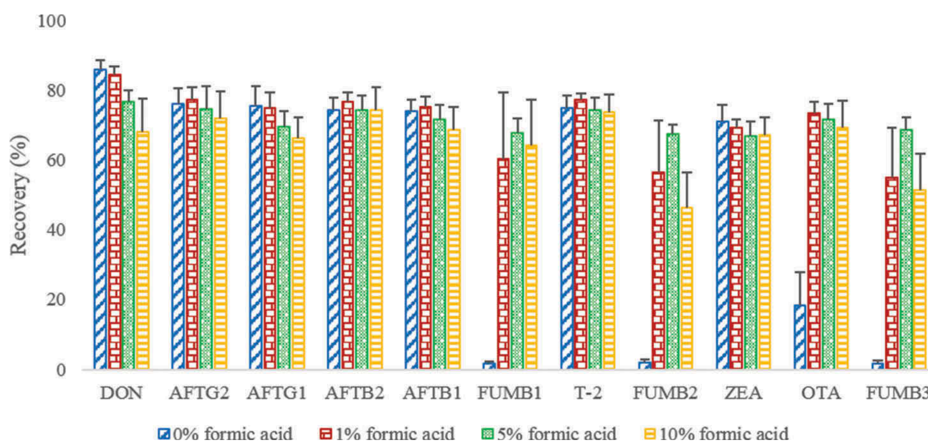

Figure 4. Comparison of the recoveries obtained using the solvent extraction method in the presence of various acid modifiers (n = 3).

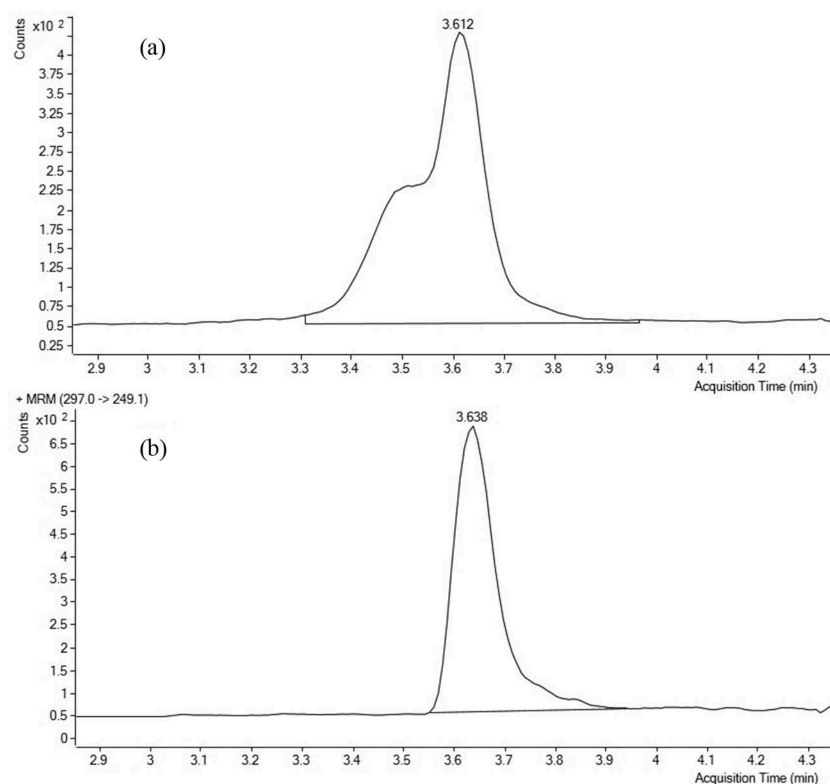

Figure 5. Extracted ion chromatograms of deoxynivalenol reconstituted with (a) 0.1% formic acid (water)/acetonitrile (50/50, v/v) and (b) water/methanol (50/50, v/v).

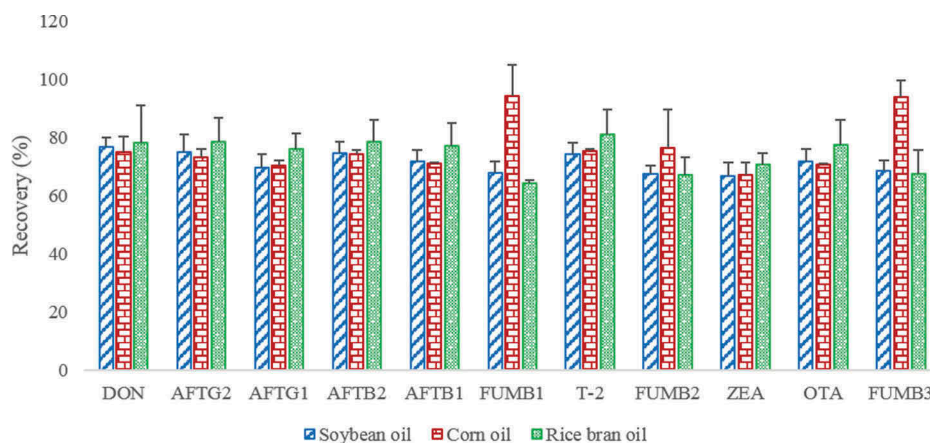

Figure 6. Recoveries from three kinds of edible oils (n = 3).

Method validation

The established method was validated in soybean oil as a representative matrix, with reference to Commission Regulation (EC) No. 401/2006 (Commission 2006). The target mycotoxins were detected without any interfering matrix peaks. The linearity was tested by analysing standard-spiked samples with the concentration range of each mycotoxin three times. The concentrations of the mycotoxins were as follows: 1, 5, 10, 50 and 100 ng/g for deoxynivalenol; 0.025, 0.25, 1.25, 2.5, 12.5 and 25 ng/g for aflatoxin B_2; 0.25, 1.25, 2.5, 12.5 and 25 ng/g for aflatoxin G_2; 0.1, 1, 5, 10, 50 and 100 ng/g for other mycotoxins. All correlation coefficients (R^2) were greater than 0.99. The recoveries were determined at low, middle, and high

concentrations within the linearity range by repeating three times; the results are described in Table 3. All mycotoxins, except zearalenone, exhibited recoveries superior to the required criteria. For zearalenone, recoveries were about 50%, slightly below the criteria where >60% is required. In the context of precision, all mycotoxins exhibited RSD values lower than the required criteria. Notably, the sensitivity of this method was equal to or greater than that of previously reported studies based on LC-MS/MS. Compared with a previous QuEChERS-based study targeting aflatoxin B_1, B_2, G_1, G_2, ochratoxin A, zearalenone and deoxynivalenol (Sharmili et al. 2016), the sensitivity of this method in the detection of mycotoxins was superior. All target mycotoxins, with the exception aflatoxin G_2, exhibited lower LOQs. Notably, the LOQ of deoxynivalenol was 2000 times lower. Also, compared with a previous MSPD-based study targeting aflatoxin B_1, B_2, G_1 and G_2 (Cavaliere et al. 2007), the LOQs of all mycotoxins were lower using our method.

Table 3. Recoveries, LOQs, and MEs of 11 target mycotoxins (matrix: soybean oil, n = 3).

Mycotoxins	Spiked conc. (ng/g)	Calculated conc. (±SD) (ng/g)	Recovery (%)	RSD (%)	LOQ (ng/g)	ME[a] (%)
DON	1	0.73 (±0.06)	72.9	7.9	1	81.74
	10	7.91 (±0.36)	79.1	4.5		
	100	77.09 (±0.66)	77.1	0.9		
AFTG$_2$	0.25	0.17 (±0.02)	68.2	11.5	0.25	77.35
	2.5	2.07 (±0.11)	82.8	5.5		
	25	19.66 (±1.02)	78.6	5.2		
AFTG$_1$	1	0.68 (±0.02)	68.2	2.4	0.1	92.49
	10	7.20 (±0.30)	72.0	4.1		
	100	75.86 (±1.56)	75.9	2.1		
AFTB$_2$	0.25	0.18 (±0.01)	73.1	5.1	0.025	96.58
	2.5	1.97 (±0.09)	78.8	4.8		
	25	19.52 (±0.28)	78.1	1.4		
AFTB$_1$	1	0.71 (±0.03)	70.7	4.9	0.1	97.57
	10	7.25 (±0.20)	72.5	2.8		
	100	75.97 (±2.03)	76.0	2.7		
FUMB$_1$	1	0.69 (±0.05)	69.5	7.2	0.1	102.49
	10	7.05 (±0.37)	70.5	5.2		
	100	64.15 (±0.48)	64.2	0.8		
T-2	1	0.78 (±0.06)	78.0	7.5	0.1	99.45
	10	7.86 (±0.21)	78.6	2.7		
	100	78.41 (±1.25)	78.4	1.6		
FUMB$_2$	1	0.71 (±0.05)	71.1	7.2	0.1	101.75
	10	6.85 (±0.31)	68.4	4.5		
	100	62.56 (±0.52)	62.6	0.8		
ZEA	1	0.60 (±0.03)	59.7	4.6	0.1	106.97
	10	5.16 (±1.04)	51.6	20.1		
	100	51.95 (±6.90)	52.0	13.3		
OTA	1	0.72 (±0.03)	71.6	4.2	0.1	102.21
	10	7.25 (±0.41)	72.5	5.6		
	100	73.61 (±1.86)	73.6	2.5		
FUMB$_3$	1	0.67 (±0.05)	66.9	8.1	0.1	101.42
	10	7.25 (±0.42)	72.5	5.8		
	100	63.25 (±0.48)	63.3	0.8		

[a]ME: peak area of matrix-matched standard/peak area of standard solution.

Matrix effects

According to a previous study (Arroyo-Manzanares et al. 2013b), the MEs of nuts and seeds in mycotoxin analysis using QuEChERS were high and up to 60.7%. Also, in the case of herbal medicines (Arroyo-Manzanares et al. 2013a), the highest ME value was 50.3% and some targets, such as aflatoxins, were even difficult to detect. Therefore, the MEs in edible oils were evaluated (see Table 3). The MEs were assessed as a percentage of the peak ratio obtained from the post-preparation extract spiked with the mycotoxin (matrix-matched standard) and the standard solution of the same concentration. Understandably, when the ME is close to 100%, the ionisation of the target compound is not affected by interfering matrix compounds. When the ME is less than 100%, the ion suppression is greater by the interfering components of sample. When the ME is higher than 100%, the ion enhancement effect is higher. Although deoxynivalenol and aflatoxin G_2, which showed peak areas of 81.74 and 77.35%, were slightly affected by ion suppression, the other mycotoxins remained relatively unaffected. From these results, we could conclude that edible oils appear to be relatively uncomplicated matrices.

Application on commercial samples

Nine edible oil samples on the market, including three soybean oil samples, three corn oil samples, and three rice bran oil samples were analysed using the established method. Zearalenone was detected in six samples and no other mycotoxins were detected. The detected concentrations of zearalenone were between 0.21 and 3.25 ng/g, and the average concentration was 0.92 ng/g, which was significantly lower than EU regulatory standards (400 ng/g).

Conclusion

A simple, rapid and sensitive method based on LC-MS/MS is proposed for the simultaneous determination of mycotoxins in edible oils. For sample preparation, solvent extraction and defatting were carried out without additional clean-up steps, which led to a fast and high-throughput analysis. The LOQs of the mycotoxins were between 0.025 and 1 ng/g. For zearalenone, the LOQ was 0.1 ng/g, which was much lower than the 400 ng/g regulatory

standard. Also, the LOQ for aflatoxin B_1 was 0.1 ng/g, which was much lower than the regulatory standard of 10–20 ng/g. The developed method could be a more efficient alternative method in terms of time and cost as compared to existing immunoaffinity columns, solid phase extraction, and other methods. In addition, the safety of several edible oils was confirmed by monitoring real samples. However, the number of samples was insufficient, so additional studies involving larger sample sizes are needed.

Disclosure statement

No potential conflict of interest was reported by the authors.

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