Appl Biol Chem (2016) 59(6):881–887
DOI 10.1007/s13765-016-0238-8

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

# Simultaneous determination of the levels of deoxynivalenol, 3-acetyldeoxynivalenol, and nivalenol in grain and feed samples from South Korea using a high-performance liquid chromatography–photodiode array detector

**Dong-Ho Kim[1,2] · Sung-Yong Hong[2] · Mi-Hyeon Jeon[1] · Jae-Min An[1] · Sung-Youn Kim[1] · Hyo-Young Kim[1] · Bo Ryun Yoon[2] · Soo Hyun Chung[2]**

Received: 26 July 2016 / Accepted: 16 November 2016 / Published online: 24 November 2016


**Abstract** An HPLC method combined with immunoaffinity clean-up was developed for the simultaneous analysis of deoxynivalenol (DON), nivalenol (NIV), and 3-acetyldeoxynivalenol (3AcDON) in grains and feeds. The limits of quantification of the method for DON, 3AcDON, and NIV were 11.0, 27.6, and 13.5 ng/g, respectively. HPLC analyses of 300 samples of grains and feeds collected in South Korea showed that the incidence of DON and NIV in corn was 22.5 and 7.5%, while that in rice was 4.0 and 54.0%, respectively. The incidence of DON and NIV in mixed grain powder was 62.0 and 24.0%, respectively. Our study also showed that animal feeds were mainly contaminated with DON (98.1%) in the range of 32.8–950.25 ng/g with the mean concentration of 353.32 ng/g. The levels of DON in grains did not exceed the maximum allowable limit (1 mg/kg) set by the Korean Food and Drug Administration.

**Keywords** 3-Acetyldeoxynivalenol · Deoxynivalenol · Feed · Grain · Nivalenol

Dong-Ho Kim and Sung-Yong Hong have contributed equally to this work.

**Electronic supplementary material** The online version of this article (doi:10.1007/s13765-016-0238-8) contains supplementary material, which is available to authorized users.

✉ Soo Hyun Chung
chungs59@korea.ac.kr

[1] National Agricultural Products Quality Management Service, 5-3 Gimcheon Innocity, Nam-myeon, Gimcheon, Gyeongsangbuk-do 39660, Republic of Korea

[2] Department of Integrated Biomedical and Life Science, Korea University, Seoul 02841, Republic of Korea

## Introduction

Mycotoxins are toxic secondary metabolites produced by fungi, and their occurrence on food and feeds has significantly increased, possibly due to global climate change (Kim et al. 2013b; Uhlig et al. 2013). Trichothecenes are toxic epoxy-sesquiterpenoid compounds; deoxynivalenol (DON) and nivalenol (NIV) are type B trichothecenes (D'Mello et al. 1999; Ok et al. 2009a). 3-Acetyldeoxynivalenol (3AcDON) is a derivative of DON and is formed by acetylation of DON at the C-3 position (Vujanovic et al. 2012). DON is produced predominantly on wheat, barley, and corn, and less often on oats, rice, rye, and sorghum by *Fusarium graminearum* (*Gibberella zeae*) and *Fusarium culmorum* (Foroud and Eudes 2009). They can cause *Fusarium* head blight on wheat and *Gibberella* ear rot on corn. Although DON is classified into Group 3 (not classifiable as to its carcinogenicity to humans) by the International Agency for Research on Cancer (IARC) of the WHO, it is water-soluble and chemically stable under most of the food processing conditions and can cause vomiting, diarrhea, weight loss, and immunotoxicity in humans and animals (IARC 2002; Larsen et al. 2004). Moreover, it can cause feed refusal and immunosuppression in animals (D'Mello et al. 1999). The FAO/WHO Joint Expert Committee on Food Additives (JECFA) set a provisional maximum tolerable daily intake (PMTDI) of 1 ng/g body weight for DON after its risk assessment was done in 2001 (JECFA 2001). However, in 2011 the JECFA newly set a group PMTDI of 1 ng/g body weight for DON and its acetylated derivatives including 3AcDON since 3AcDON can be converted to DON in vivo and can exhibit toxicity (JECFA 2011). At least 37 countries regulate DON at levels of 300–2000 ng/g as a maximum allowable limit

(FAO 2004). The Korean Food and Drug Administration (KFDA) has set legal limits of 1, 2, and 0.5 μg/g for DON in grains and their products, corn and their products, and cereals, respectively (KFDA 2010; Ok et al. 2009b).

NIV is similar to DON in its chemical structure and properties; they can be simultaneously detected in grains. NIV is produced by *Fusarium nivale*, *F. graminearum*, and *F. culmorum* (Grabarkiewicz-Szczesna et al. 2001). It can cause emesis, diarrhea, necrotic lesions of the skin, and hemorrhage of tissues in lungs and intestines, and damage immune functions. In addition, it can inhibit DNA synthesis (Bretz et al. 2005). The European Commission (EC) established a PMTDI of 0.7 ng/g body weight/day for NIV after its toxicity assessment (Ryu et al. 1988; Ohtsubo et al. 1989; Eudes et al. 2000; Kim 2010). However, the maximum allowable legal limit of NIV has not yet been set in South Korea, and effective measures and research are required for the establishment of a legal limit. Thus, the Codex Committee on Contaminants in Foods began a review for total regulation of DON and its derivatives such as 3AcDON (KFDA 2010).

Moreover, only one previous study has reported levels of DON, NIV, and 3AcDON in samples collected from South Korea. The authors of that study measured the levels of the mycotoxins in the samples by gas chromatography (GC) combined with an extraction method using less specific solid-phase cartridges against DON, NIV, and 3AcDON for the cleaned-up samples (Ok et al. 2011). The GC method for determination of the levels of mycotoxins in samples also includes inconvenient and cumbersome steps for derivatization of the samples. In addition, the study reported levels of the toxins only in human dietary samples.

Thus, in this study we established a method for simultaneous analyses of DON, NIV, and 3AcDON in grain and feed samples by high-performance liquid chromatography (HPLC). Furthermore, we determined the levels of DON, NIV, and 3AcDON contamination in grains and feeds using the improved method. To the best of our knowledge, this is the first report on the establishment of a method for simultaneous determination and monitoring of DON, 3AcDON, and NIV in grains and animal feeds collected in South Korea.

## Materials and methods

### Chemicals and reagents

The standards of DON, 3AcDON, and NIV were obtained from Romer Labs (Union, MO, USA). Acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). Immunoaffinity columns (IAC; DON–NIV) were obtained from VICAM (Milford, MA, USA). The phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich [St. Louis, MO, USA]) was used for pH adjustment of toxin extracts. Ultrapure water was supplied by Barnstead ultrapure water purification system (Barnstead Thermolyne, Dubuque, IA, USA).

### Standard solutions

Each stock solution (10 mg/L) of the toxin standards was prepared by mixing 100 μL of each standard (100 mg/L) with 900 μL of 15% ACN (ACN:water = 15:85, v/v). A series of standard solutions for each toxin (20, 100, 250, 500, and 1000 ng/mL) were prepared freshly by dilutions of each stock solution with 15% ACN. The toxin standard solutions were used to assess the linearity of a series of DON, 3AcDON, or NIV concentrations by HPLC–photodiode array (HPLC–PDA). Each standard solution was injected into the HPLC–PDA in triplicate. In addition, a toxin mixture including DON, 3AcDON, and NIV at 1000 ng/mL of each toxin concentration was used as a mixed standard solution for optimization of HPLC–PDA conditions.

### Commercial products

Three types of grains (40 maize samples, 50 polished rice samples, and 50 mixed grain powder [MGP] samples) were purchased from local markets in South Korea in 2014. Three types of commercial animal feeds (50 chicken feeds, 60 cattle feeds, and 50 pig feeds) were collected from retail markets. Approximately 1 kg of each type of sample was stored at −18 °C before analysis.

### Organic solvent selection for toxin extraction from samples

In order to select proper solvents for extraction of the three different toxins from samples, we measured the recovery rates of toxins in polished rice, which was spiked with 500 ng/g of each type of toxin standard solution, using different concentrations of ACN and MeOH (25 and 75% ACN, and 30, 50, and 70% MeOH).

### Extraction of toxins in samples and immunoaffinity column clean-up

Twenty grams of each sample was weighed and placed in a 250-mL of Erlenmeyer flask after being ground into powder with a food grinder (Hallde, KISTA, Sweden). One hundred milliliters of 25% ACN as a selected solvent was added to it and toxins were extracted by shaking at 320 rpm for 1 h with a shaker (EYELA, Tokyo, Japan).

**Table 1** Efficiency of the extraction of three mycotoxins in polished rice using different percentages of two solvents

| Mycotoxin | Spiked level (ng/g) | Recovery[a] (%) | | | | |
|---|---|---|---|---|---|---|
| | | 30% MeOH | 50% MeOH | 70% MeOH | 25% ACN | 75% ACN |
| DON ($n = 3$) | 500 | 70 ± 5.07 | 57 ± 0.84 | 78 ± 1.15 | 84 ± 4.71 | 58 ± 2.42 |
| 3AcDON ($n = 3$) | 500 | 93 ± 9.17 | 133 ± 0.24 | 101 ± 0.60 | 108 ± 0.29 | 101 ± 3.03 |
| NIV ($n = 3$) | 500 | 29 ± 2.45 | 31 ± 0.37 | 47 ± 4.89 | 99 ± 3.71 | 28 ± 0.64 |

[a] Data are presented as mean ± standard deviation

After the extract was centrifuged at 3000 rpm for 5 min at 4 °C, the supernatant was filtered through Whatman No. 4 filter paper. Ten milliliters of each filtrate was diluted with 40 mL of PBS and then filtered through Whatman GF/A glass microfiber filter paper. Twenty-five milliliters of the filtrate was loaded onto an immunoaffinity column (IAC: DON–NIV) and passed through at a flow rate of 1 drop/s. The column was washed with 25 mL of pure water until 2–3 mL of air passed through it, and toxins were finally eluted from the column with 3 mL of ACN. The eluates were evaporated to dryness under a gentle stream of $N_2$ at 45 °C, and the residues were redissolved in 0.5 mL of 15% ACN. The solutions were vortexed for 30 s and filtered through a 0.22-μm syringe filter. Fifty microliters of each filtrate was injected into the HPLC–PDA system.

### Preparation of samples spiked with mixed standard solutions including DON, 3AcDON, and NIV

In order to determine the accuracy of the method, polished rice and animal feeds that were naturally uncontaminated with toxins were spiked with a mixed standard solution including DON, 3AcDON, and NIV to give concentrations of 250 ng/g in the spiked samples. Extraction and clean-up of analytes from the spiked samples were performed in triplicate by the procedures described above. The accuracy of the methods was evaluated by the recovery of the three toxins obtained from samples fortified with known contents of the toxin standard solutions. The recoveries were calculated by the following equation:

$$\text{Recovery} = \frac{\text{Each toxin concentration measured from the spiked sample} \times 100}{\text{Each toxin concentration used for spiking the sample}}$$

### HPLC–PDA conditions

An HPLC (Shiseido NASCA2 UHPLC System, Tokyo, Japan) equipped with a PDA detector was used to detect DON, 3AcDON, and NIV, and the determination of the three analytes was performed at an absorption wavelength of 220 nm. Separation was carried out on a Capcell Pak UG120 C18 column (4.6 mm × 250 mm, 5 μm particle size, Shiseido, Japan), and the oven temperature was set at 35 °C. The mobile phase, 15% ACN (ACN:water = 15:85, v/v), was pumped at a flow rate of 0.7 mL/min. A gradient elution program was applied as follows: after 15% ACN was maintained for 6 min, it linearly increased from 15% at 6 min to 30% at 9.0 min. It was then held at 30% from 9.0 to 15.0 min and linearly decreased from 30% at 15.0 min to 15% at 17 min. Subsequently, 15% ACN was held for 3 min for re-equilibration of the column before injection of the next sample, giving a total run time of 20 min. The injection volume of the samples was 50 μL.

## Results

### Extraction of DON, 3AcDON, and NIV from samples

The best selection of solvents for mycotoxin extraction is one of the most important factors for good recoveries of toxins. For the extraction of trichothecenes such as DON, 3AcDON, and NIV from the samples, an ACN/water mixture and an MeOH/water mixture have been widely used (Juan et al. 2012; Rasmussen et al. 2012). Thus, the conditions for the extraction of DON, 3AcDON, and NIV from

**Table 2** Recoveries and RSDs of three mycotoxins in rice and animal feed samples

| Mycotoxin | Spiked level (ng/g) | Recovery in rice (%) | RSD[a] in rice (%) | Recovery in feed (%) | RSD[a] in feed (%) |
|---|---|---|---|---|---|
| DON ($n = 3$) | 250 | 102 | 10.7 | 131 | 4.5 |
| 3AcDON ($n = 3$) | 250 | 109 | 11.4 | 93 | 4.2 |
| NIV ($n = 3$) | 250 | 99 | 10.6 | 89 | 8.1 |

[a] RSD indicates relative standard deviation

**Table 3** Incidence and levels of three mycotoxins in samples

| Sample | DON | | | NIV | | | 3AcDON | | |
|---|---|---|---|---|---|---|---|---|---|
| | Incidence (%) | Range (ng/g) | Mean[b] (ng/g) | Incidence (%) | Range (ng/g) | Mean[b] (ng/g) | Incidence (%) | Range (ng/g) | Mean[b] (ng/g) |
| Cattle feed ($n = 60$) | 100.0 | 91.65–950.25 | 602.51 | 28.3 | 0–111.52 | 57.26 | 3.3 | 0–52.10 | 32.75 |
| Chicken feed ($n = 50$) | 94.0 | 0–603.10 | 258.36 | 8.0 | 0–101.23 | 63.25 | 2.0 | 0–29.70 | 29.70 |
| Pig feed ($n = 50$) | 100.0 | 32.28–932.48 | 164.74 | 22.0 | 0– 84.21 | 34.78 | 0 | 0 | 0 |
| Corn ($n = 40$) | 22.5 | 0–232.56 | 190.78 | 7.5 | 0–181.41 | 135.39 | 0 | 0 | 0 |
| Polished rice ($n = 50$) | 4.0 | 0–75.30 | 52.75 | 54.0 | 0–214.51 | 132.24 | 0 | 0 | 0 |
| MGP[a] ($n = 50$) | 62.0 | 0–125.47 | 35.73 | 24.0 | 0–198.70 | 41.62 | 0 | 0 | 0 |

[a] MGP represents mixed grain powder

[b] Mean indicates an average in positive samples

the samples were optimized in this study. We measured the recovery rates of the mycotoxins in rice, which was spiked with 500 ng/g of each type of toxin, using different concentrations of ACN and MeOH (25 and 75% ACN, and 30, 50, and 70% MeOH). All five solvents showed good recoveries (93–133%) only in the extraction of 3AcDON, while 25% ACN showed high recoveries in the extraction of DON (84%) and NIV (99%) as well as 3AcDON (108%) (Table 1). Therefore, we selected 25% ACN as a solvent for the extraction of mycotoxins from the samples.

## Validation of the analytical methods

The choice of a mobile phase in HPLC analyses is very important for the high separation efficiency of DON, 3AcDON, and NIV. We utilized different ratios of ACN to water to select a mobile phase for good separation of mycotoxins by HPLC. A mixture of ACN and water (ACN:water = 15:85, v/v) gave the best results for the separation of all three toxins with a flow rate of 0.7 mL/min (data not shown).

The analytical method using HPLC–PDA was validated using parameters such as linearity, sensitivity, and accuracy. The linearity of a series of DON, 3AcDON, and NIV concentrations in the analytical method was assessed by each standard curve using five levels of each toxin standard solution: 20, 100, 250, 500, and 1000 ng/mL. The calibration curve for each toxin was constructed by plotting the peak areas ($y$-axis) versus toxin concentrations ($x$-axis) in the HPLC analyses. The linearity was determined by linear regression analysis. The curves for the three different mycotoxins showed $r^2$ values (correlation coefficient) in the range of 0.9995–0.9999 (Fig. S1 A, B, and C). Therefore, we concluded that the calibration curves for all three mycotoxins were linear in the range of 20–1000 ng/mL.

The sensitivity of the analytical method using HPLC–PDA was determined by a limit of detection (LOD) and a limit of quantification (LOQ). They were calculated as the signal-to-noise (S/N) ratios of 3 and 10, respectively, which were determined using HPLC software (Shiseido EZChrom Elite system). The LODs of the analytical method for DON, 3AcDON, and NIV were 3.3, 8.3, and 4.0 ng/g, whereas the LOQs of the method for the mycotoxins were 11.0, 27.6, and 13.5 ng/g, respectively. The levels of LOD and LOQ of the method for all of the toxins were as low as those for the detection of trace amounts of the toxins, indicating that the method is highly sensitive in the determination of all three mycotoxins.

The accuracy of the analytical method was evaluated by the recoveries for DON, 3AcDON, and NIV obtained from the samples fortified with each type of toxin. The recoveries were calculated as described in materials and methods. They were measured by injecting toxins extracted from naturally uncontaminated polished rice and animal feed samples, which were spiked with 250 ng/g of each type of toxin solution, into the HPLC–PDA. The recovery rates for DON, 3AcDON, and NIV in polished rice were 102, 109, and 99% along with the relative standard deviations (RSDs) of 10.7, 11.4, and 10.6%, while those in animal feeds were 131, 93, and 89% along with the RSDs of 4.5, 4.2, and 8.1%, respectively (Table 2). Overall, the recovery rates in all of the samples were higher than 89% and the RSD values were less than 11.4%. The EC has set the allowable limits of the RSD and recovery in the analytical method for DON at less than 20% of RSD and 60–110% of recovery rates, respectively, in food samples contaminated with 100–500 ng/g of DON (EC 2006). Our results showed that the RSD and recoveries from polished rice and feeds in this study satisfy the allowable limits of those recommended for DON by the EC. Thus, we concluded that the analytical method had good recoveries from the matrices of rice and feeds. Overall, the analytical method established in this study showed good linearity, sensitivity, specificity, and accuracy in the determination of mycotoxins by HPLC–PDA.

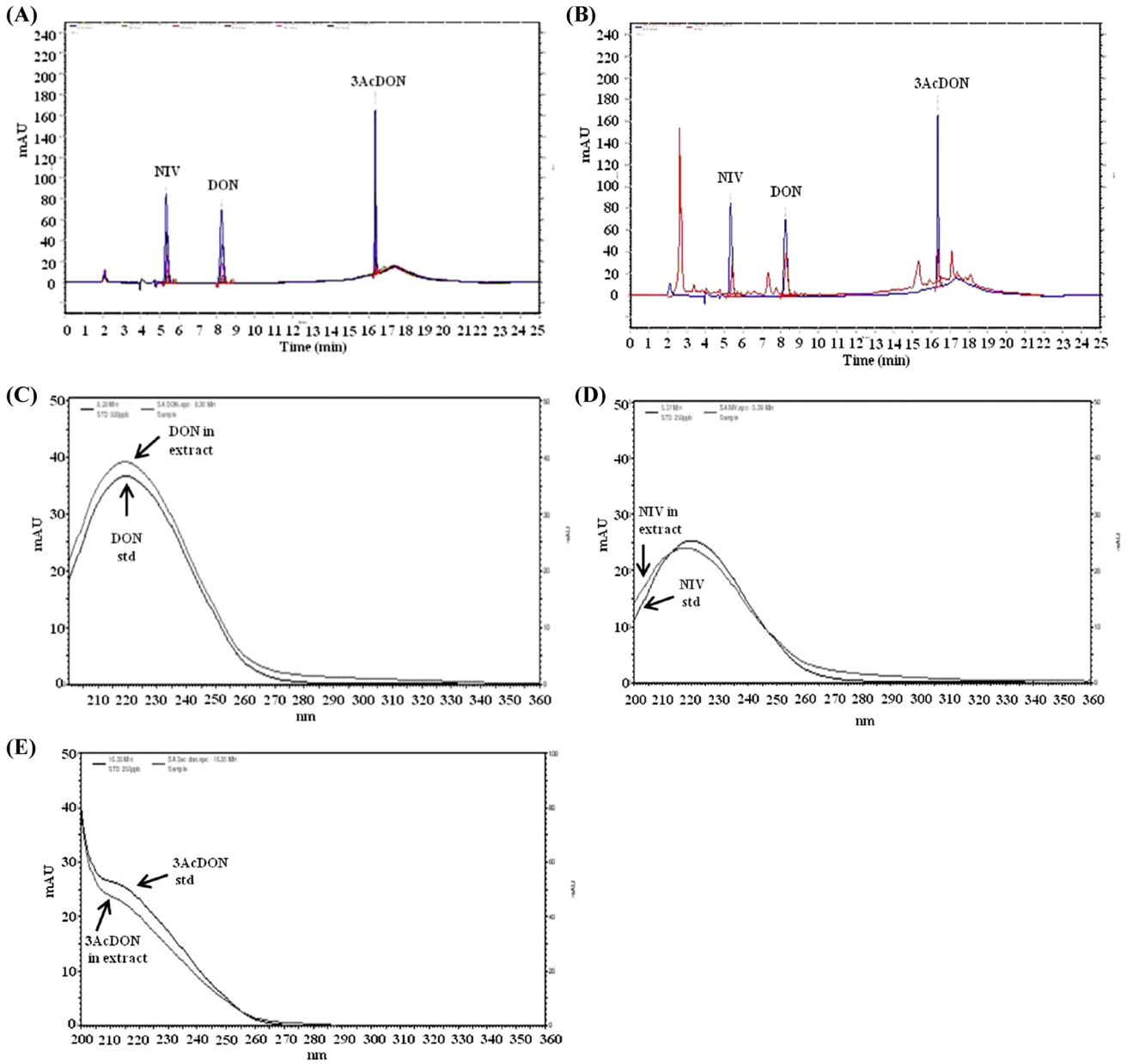


**Fig. 1** Chromatograms of DON, 3AcDON, and NIV using HPLC–PDA. (**A**) Chromatogram of DON, 3AcDON, and NIV in a series of standard solutions. Toxins in each standard solution were injected at the levels of 20, 50, 100, 250, 500, and 1000 ng/mL into the HPLC–PDA system. The retention times of peaks corresponding to each toxin are as follows: NIV, 5.3 min; DON, 8.2 min; and 3AcDON, 16.3 min. (**B**) Chromatogram of DON, 3AcDON, and NIV extracted from chicken feeds. Fifty microliters of toxin extract from chicken feeds was injected into the HPLC–PDA system. The chromatogram was overlapped with that of 1000 ng/mL of a standard solution. The retention times of peaks corresponding to each toxin are as follows: NIV, 5.3 min; DON, 8.2 min; and 3AcDON, 16.3 min. (**C**–**E**) UV absorption spectrum of DON, NIV, or 3AcDON extracted from chicken feeds, respectively. Fifty microliters of toxin extract from chicken feeds was injected into the HPLC–PDA system. Each spectrum was overlapped with that of 500 ng/mL of DON or 250 ng/mL of NIV or 3AcDON standard solution. std: standard

## Monitoring the levels of DON, 3AcDON, and NIV in commercial products

The analytical method validated above was used for the determination of DON, 3AcDON, and NIV in three types of grains (40 maize samples, 50 polished rice samples, and 50 MGP samples) and animal feeds (50 chicken feeds, 60 cattle feeds, and 50 pig feeds) collected from local markets in South Korea. The occurrence and levels of all three toxins in the commercial products are summarized in

Table 3. Figure 1 shows the representative chromatograms and UV absorption spectrums of DON, 3AcDON, and NIV extracted from chicken feeds and in standard solutions. The analytical method established in this study did not produce any peaks that interfered with those of the three mycotoxins within 20 min of retention time in chromatograms of the samples as shown in Fig. 1. The 3AcDON was not detected in any type of grains and pig feed samples, whereas NIV and DON were detected in all types of grains and feed samples. These results are in agreement with the those from a previous report on human dietary cereal samples, in which the authors detected very low levels of 3AcDON in corn and mixed grains (4.1 and 4.7 ng/g, respectively) compared to those of DON and NIV (Ok et al. 2011). Also, in our study DON was detected in all of the cattle and pig feed samples, 94% of the chicken feed samples, 22.5% of corn, 62.0% of MGP, and 4.0% of polished rice. Samples in which the highest level of DON (950.25 ng/g) was detected were animal feeds, which indicates that DON contamination is most serious and prevalent in animal feeds in South Korea. Kim et al. (2013a) showed that DON was detected in rice plants but not in polished rice. The results in our current study are in agreement with their results. We detected DON in only 4% of polished rice as shown in Table 3, but NIV was detected in 54% of the samples. Furthermore, other researchers have reported that the levels of NIV were higher than those of DON in wheat contaminated with trichothecenes when analyzed using HPLC (Lee et al. 1987; Sohn et al. 1998). Their results are consistent with our results in which the levels of NIV (132.24 ng/g of the mean concentration) were higher than those of DON (52.75 ng/g of the mean concentration) in polished rice (Table 3). In contrast, for animal feeds we detected higher occurrences and levels of DON (98.1%, 353.32 ng/g of the mean concentration) than those of NIV (10.0%, 13.75 ng/g of the mean concentration), which is in agreement with the data from European countries (EFSA 2013).

## Discussion

In this study, we validated the analytical method using the parameters such as linearity, sensitivity, and accuracy by HPLC–PDA. The analytical method showed good linearity, sensitivity, specificity, and accuracy in the determination of the three types of trichothecenes. In addition, simultaneous determination of the levels of DON, 3AcDON, and NIV in three types of grains and animal feeds collected from South Korea was performed using the analytical method established in the present study. Monitoring the levels of DON, 3AcDON, and NIV in grains and feeds demonstrated that DON contamination is most serious and prevalent in animal feeds in South Korea.

Moreover, in our current study, the levels of DON detected in grains satisfied the maximum allowable limit (1 mg/kg for DON in grains and their products) set by the KFDA in South Korea (KFDA 2010; Ok et al. 2009b). In addition, the levels of DON detected in feeds also satisfied the maximum allowable limit set for grains by the KFDA when the limit of DON for grains is used because a limit for feeds has not been established in South Korea. Furthermore, the levels of NIV and 3AcDON in grains and feeds were below the maximum allowable legal limit of DON set by the KFDA when the limit of DON is used for comparison since the legal limits of NIV and 3AcDON are not yet set in South Korea. Overall, although the occurrence and levels of NIV and 3AcDON in grains and feeds were relatively low in our study, more extensive and active research is required for the establishment of the legal limits of NIV and 3AcDON in South Korea. In addition, monitoring of the levels of DON in grain and feeds should be continued because it can cause toxic responses such as immunotoxicity in humans and animals.

**Acknowledgements** The authors would like to thank the financial support provided by the National Agricultural Products Quality Management Service in South Korea.

## References

Bretz M, Knecht A, Gockler S, Humpf HU (2005) Structural elucidation and analysis of thermal degradation products of the *Fusarium* mycotoxin nivalenol. Mol Nutr Food Res 49:309–316

D'Mello JPF, Placinta CM, Macdonald AMC (1999) *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. Anim Feed Sci Technol 80:183–205

EC (2006) Commission Regulation 401/2006 of 23 February 2006. Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off J Eur Union L70:12–34

EFSA (2013) Deoxynivalenol in food and feed: occurrence and exposure. EFSA J 11:3379

Eudes F, Comeau A, Rioux S, Collin J (2000) Phytotoxicity of eight mycotoxins associated with *Fusarium* in wheat head blight. Can J Plant Pathol 22:286–292

FAO (2004) Worldwide regulations for mycotoxins in food and feed in (2003) FAO food and nutrition paper 81, vol 81. Food and Agriculture Organization, Rome

Foroud NA, Eudes F (2009) Trichothecenes in cereal grains. Int J Mol Sci 10:147–173

Grabarkiewicz-Szczesna J, Kostecki M, Golinski P, Kiecana I (2001) Fusariotoxins in kernels of winter wheat cultivars field samples collected during 1993 in Poland. Die Nahr 45:28–30

IARC (2002) Monograph on the evaluation of carcinogenic risk to humans, vol 82. World Health Organization, Lyon

JECFA (2001) Joint FAO/WHO Expert Committee on Food Additives. Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth meeting of JECFA. WHO food additives series 47/FAO food and nutrition 74, vol 47. World Health Organization, Geneva

JECFA (2011) Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain contaminants in food. Prepared by the seventy-second report of the JECFA. WHO technical report series 959, vol 959. World Health Organization, Geneva

Juan C, Ritieni A, Manes J (2012) Determination of trichothecenes and zearalenones in grain cereal, flour and bread by liquid chromatography tandem mass spectrometry. Food Chem 134:2389–2397

KFDA (2010) Food standards codex. Korea Foods Industry Association, Seoul

Kim YK (2010) Development and application of an analytical method for the simultaneous determination of deoxynivalenol and nivalenol in domestic brown and polished rice. Chungbuk National University

Kim DH, Jang HS, Choi GI, Kim HJ, Kim HL, Cho HJ, Lee C (2013a) Occurrence of mycotoxins in Korean grains and their simultaneous analysis. Korean J Food Sci Technol 45:111–119

Kim YS, Kim YS, Kim MG, Lee SB, Lee JY, Oh SH, Jung YJ, Seo MY, Sung JH, Lee W, Lee JB, Yoon MH (2013b) The safety assessment of aflatoxins and deoxynivalenol in cereals and their products. J Food Hyg Saf 28:158–167

Larsen JC, Hunt J, Perrin I, Ruckenbauer P (2004) Workshop on trichothecenes with a focus on DON: summary report. Toxicol Lett 153:1–22

Lee US, Jang HS, Tanaka T, Oh YJ, Cho CM, Ueno Y (1987) Effect of milling on decontamination of *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone in Korean wheat. J Agric Food Chem 35:126–129

Ohtsubo K, Ryu JC, Nakamura K, Izumiyama N, Tanaka T, Yamamura H, Kobayashi T, Ueno Y (1989) Chronic toxicity of nivalenol in female mice: a 2-year feeding study with *Fusarium nivale* Fn 2B-moulded rice. Food Chem Toxicol 27:591–598

Ok HE, Chang HJ, Ahn JH, Cho JY, Chun HS (2009a) Estimation of measurement uncertainty for the HPLC analysis of deoxynivalenol in wheat. Korean J Food Sci Technol 41:258–264

Ok HE, Chang HJ, Choi SW, Cho TY, Oh KS, Chun HS (2009b) Occurrence and intake of deoxynivalenol in cereal-based products marketed in Korea during 2007–2008. Food Addit Contam Part B Surveill 2:154–161

Ok HE, Choi SW, Chung SH, Kang YW, Kim DS, Chun HS (2011) Natural occurrence of type-B trichothecene mycotoxins in Korean cereal-based products. Food Addit Contam Part B Surveill 4:132–140

Rasmussen PH, Nielsen KF, Ghorbani F, Spliid NH, Nielsen GC, Jorgensen LN (2012) Occurrence of different trichothecenes and deoxynivalenol-3-beta-D-glucoside in naturally and artificially contaminated Danish cereal grains and whole maize plants. Mycotoxin Res 28:181–190

Ryu JC, Ohtsubo K, Izumiyama N, Nakamura K, Tanaka T, Yamamura H, Ueno Y (1988) The acute and chronic toxicities of nivalenol in mice. Fundam Appl Toxicol 11:38–47

Sohn DH, Lee HB, Kwak BY, Kim SH, Kweon CH (1998) Enzyme-linked immunosorbent assay for detection of nivalenol. J Food Hyg Saf 13:129–134

Uhlig S, Eriksen GS, Hofgaard IS, Krska R, Beltran E, Sulyok M (2013) Faces of a changing climate: semi-quantitative multi-mycotoxin analysis of grain grown in exceptional climatic conditions in Norway. Toxins (Basel) 5:1682–1697

Vujanovic V, Goh YK, Daida P (2012) Heat- and cold-shock responses in *Fusarium graminearum* 3 acetyl- and 15 acetyl-deoxynivalenol chemotypes. J Microbiol 50:97–102