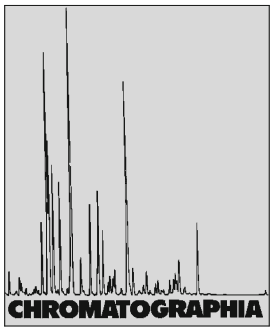
Simultaneous Determination of Deoxynivalenol and Nivalenol in Traditional Chinese Medicine by SPE and LC



2010, *72*, 551–555

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Received: 14 April 2010 / Revised: 13 June 2010 / Accepted: 15 June 2010
Online publication: 2 July 2010

Abstract

A liquid chromatography method for simultaneous determination of deoxynivalenol (DON) and nivalenol (NIV) in traditional Chinese medicine (TCM) is described for the first time. Different clean-up columns and extraction solvents have been optimized. The DON purification column was chosen as the best. The detection limits for DON and NIV were 62.5 and 50.0 μg kg^{-1}, respectively. Recoveries from different TCMs spiked with DON and NIV at levels ranging from 0.5 to 10 mg kg^{-1} were 82.8–99.9% and 80.8–100.3%, respectively. None of the 30 commercially available TCM samples analyzed, were found to contain any detectable amounts of DON and NIV.

Keywords

Column liquid chromatography
UV detection
Deoxynivalenol purification column
Traditional Chinese medicine
Deoxynivalenol and nivalenol

Introduction

The occurrence of trichothecenes in agricultural commodities is a major health concern for animal and humans. The most prominent genera of fungi producing trichothecenes are *Fusarium*, *Myrothecium*, *Trichoderma*, and *Stachybotrys* [1, 2]. Trichothecenes are capable of producing a wide range of toxic effects. The clinical symptoms of intoxicated animals include feed refusal, emesis, nervous system disturbance, depletion of hepatic glycogen, changes in blood glucose concentration, irritation of the gastrointestinal tract, diarrhoea, reduced weight gains, inhibition of protein and DNA synthesis, immunotoxic effects, alimentary toxic aleukia, and hemorrhaging [3, 4].

Deoxynivalenol (DON) and nivalenol (NIV) are naturally occurring type B trichothecene mycotoxins produced by *Fusarium* genus, in particular, *F. graminearum*, *F. culmorum* and *F. poae* [5]. DON is the most common trichothecene, followed by nivalenol, T-2 toxin and HT-2 toxin. Wheat, barley, oats, rye, and maize used for human and animal consumption are frequently contaminated with DON and NIV. The natural occurrence of DON and NIV in cereals and cereal-based products has been reported in Germany, Russia, China, Brazil, etc. [6–8]. Due to the high toxicity and the widespread distribution of trichothecenes, the European Commission enforced the limits of DON with levels of 200–1,750 μg kg^{-1} in cereals and cereal-based products [9]. No legal limits have yet been provided for NIV, although it is well known that cereal grains and animal feeds could be frequently contaminated.

Many different methods for the determination of DON and NIV have been established based on thin-layer chromatography (TLC) [10], enzyme-linked immunosorbent assay (ELISA) [11, 12], gas chromatography (GC) with

Full Short Communication
DOI: 10.1365/s10337-010-1679-6
0009-5893/10/09

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Table 1. The analysis results of DON and NIV in 30 TCMs

Sample name	Place of purchase	Contents of DON (μg kg^{-1})	Contents of NIV (μg kg^{-1})
Semen Coicis (lot:080201)	Henan	ND	ND
Semen Coicis (lot:080202)	Beijing	ND	ND
Semen Coicis (lot:080203)	Beijing	ND	ND
Semen Coicis (lot:080204)	Beijing	ND	ND
Semen Coicis (lot:080205)	Beijing	ND	ND
Semen Coicis (lot:080206)	Beijing	ND	ND
Semen Coicis (lot:080207)	Beijing	ND	ND
Semen Coicis (lot:080208)	Beijing	ND	ND
Radix Notoginseng	Beijing	ND	ND
Fructus Hordei Germinatus	Beijing	ND	ND
Massa Medicata Fermentata	Beijing	ND	ND
Radix Glycyrrhizae	Beijing	ND	ND
Fructus Foeniculi	Beijing	ND	ND
American Ginseng	Beijing	ND	ND
Semen Nelumbinis	Beijing	ND	ND
Lycium chinense Mill.	Beijing	ND	ND
Fujian Massa Medicata Fermentata	Beijing	ND	ND
Rhizoma Chuanxiong	Beijing	ND	ND
Herba Menthae	Beijing	ND	ND
Radix Angelicae Sinensis	Beijing	ND	ND
Millet sprout	Beijing	ND	ND
Fructus Crataegi	Beijing	ND	ND
Radix Bupleuri	Beijing	ND	ND
Radix Isatidis	Beijing	ND	ND
Radix Morindae Officinalis	Beijing	ND	ND
Radix Codonopsis	Beijing	ND	ND
Radix Polygalae	Beijing	ND	ND
Semen Persicae	Beijing	ND	ND
Radix Platycodi	Beijing	ND	ND
Cortex Magnoliae Officinalis	Beijing	ND	ND

ND not detected

Table 2. The influences of the extraction solvent and clean-up column on the recoveries spiked with DON and NIV at level of 5.0 mg kg^{-1} in black Radix Notoginseng ($n = 3$)

Influencing factor	Recovery of DON (%)	Recovery of NIV (%)
Extraction solvent		
Acetonitrile–water (84:16, *v*/*v*)	89.3	70.4
Acetonitrile–water (80:20, *v*/*v*)	98.4	82.6
Acetonitrile–water (70:30, *v*/*v*)	94.0	82.2
Methanol–water (80:20, *v*/*v*)	55.0	75.9
Methanol–water (70:30, *v*/*v*)	30.3	65.6
Clean-up column		
Mycosep 225 column	94.8	56.0
Bond Elut Mycotoxin column	90.5	60.1
Puri Tox^{SR} TC-M160 column	97.4	82.2
Puri Tox^{SR} TC-T220 column	93.1	68.8
Puri Tox^{SR} TC-T200 DON purification column	98.4	83.8

electron-capture detection (ECD) or mass spectrometry (MS) [13–15], liquid chromatography (LC) and LC–MS [16–19]. However, all analytical methods for the determination of DON and NIV described above have been concentrated mainly on agricultural products, foods and feeds. Recently, it was reported that 62% of the 84 medicinal and aromatic herb samples collected in Spain contaminated with DON were determined by ELISA after a clean-up step with multifunctional columns [20]. In addition, out of 58 medicinal herbs and related products samples in China, two samples of one medicinal herb, Semen Coicis, and one sample of Chinese patent medicine, Baohe pills, contaminated with DON were determined using GC-ECD after an immunoaffinity column clean-up [21]. Although, the most significant advantage of ELISA is the low cost of each analysis, this technique is only a semi-quantitative detection method. Application of GC-ECD provides desired selectivity and sensitivity for analyses, but derivatization prior to GC is necessary. Multifunctional and immunoaffinity columns are expensive. Nowadays, more than 1,000 medicinal herbs are available on the market in China. Some medicinal herbals, for example, Semen Nelumbinis and Fructus Crataegi, are also used as food in China, and their daily consumption is 10–200 g. The medicinal herbs contain many substances, such as: flavone, saponin, alkaloid, tannin, etc. Therefore, it is important to develop an economical and efficient method for routine analysis for monitoring the levels of DON and NIV in traditional Chinese medicine (TCM).

In this paper we developed a simple and accurate method for simultaneous determination of DON and NIV in TCM using DON purification column clean-up and LC-UV. Different clean-up columns and extraction solvents have been optimized.

Experimental

Chemicals and Materials

DON and NIV were from Wako (Shanghai, China). Mycosep 225 column was from Romer Labs (Washington, USA). Bond-Elut Mycotoxin was from Varian (Harbor City, CA, USA). Puri Tox^{SR} TC-M160, Puri Tox^{SR} TC-T220 and Puri Tox^{SR} TC-T200 DON purification columns were from Trilogy Analytical Laboratory (Washington, USA). Acetonitrile and methanol were from Fisher Scientific (Waltham, USA). Other reagents were analytical grade and water was double-distilled.

Apparatus

The LC apparatus consisted of a liquid chromatography system and aUV detector. The rotary evaporator was Yarong RE-52AA (Shanghai, China). The GF-1 high-speed blender was from Qilin Company (Haimen, China).

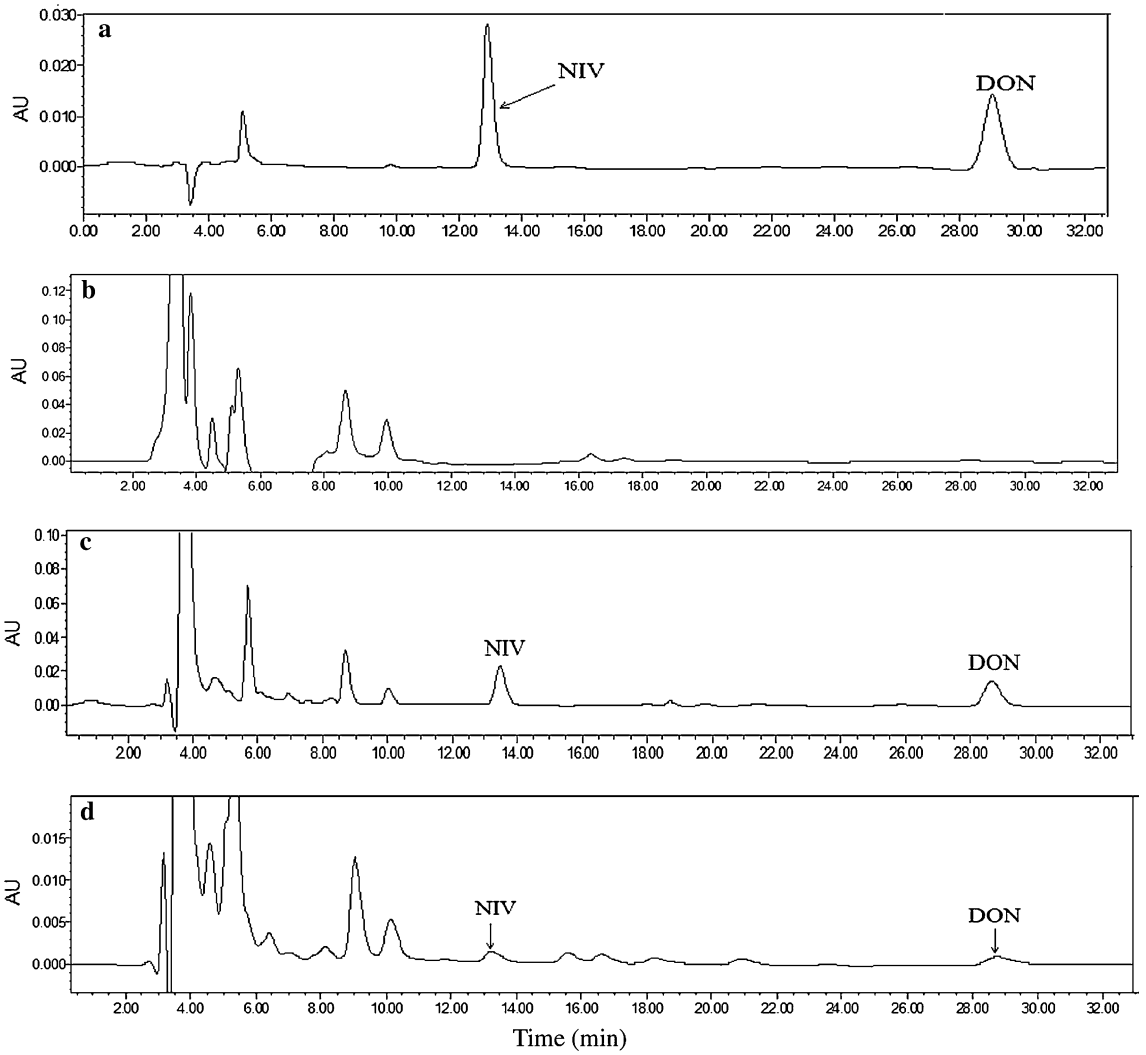

Fig. 1. Chromatograms from the LC-UV determination. **a** Standard of 0.5 μg each of DON and NIV. **b** Blank Fructus Hordei Germinatus. **c** Blank Fructus Hordei Germinatus spiked with DON and NIV at level of 10 mg kg^{-1}. **d** Blank Fructus Hordei Germinatus spiked with DON and NIV at level of 0.5 mg kg^{-1}

The analytical column was an Agela Venusil MP C_{18} column (250 mm × 4.6 mm, 5 μm) (Agela, Beijing, China), and the column temperature was kept at 25 °C. The mobile phase consisted of water–acetonitrile–methanol (90:5:5, *v*/*v*/*v*) with a flow-rate of 0.8 mL min^{-1}. DON and NIV were simultaneously detected at 218 nm. The sample volume injected was 50 μL.

Preparation of Standard Solution

A stock solution containing DON and NIV standards was prepared by dissolving the solid commercial toxins in acetonitrile to a final concentration of 100 μg mL^{-1}, and then diluting the mixture stock solution to appropriate concentrations for LC calibration curves and spiking tests.

Origin of Samples

30 samples of TCMs were purchased from the market in China. These widely used samples were selected mainly according to their medicinal parts: root, leaf, seed, etc., and some of them are used as food in China. They were stored at 4 °C prior to analysis. The samples (shown in Table 1) were identified by Professor Bengang Zhang, Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College.

Sample Extraction and Clean-Up

Samples were ground to particles of less than 1 mm with a laboratory mill, mixed and stored at −20 °C. Samples of TCM (20 g) were extracted with 100 mL acetonitrile:water (80:20, *v*/*v*) in a 250 mL screw-capped bottle by blending at high speed for 2.0 min. The extracts were filtered and 4 mL then transferred into the test tube of Puri Tox^{SR} TC-T200 DON purification column. The purification

Table 3. Recoveries from blank TCMs spiked with DON and NIV at different levels ($n = 3$)

TCMs	Spiking level (mg kg^{-1})	Recovery of DON, RSD (%)	Recovery of NIV, RSD (%)
RN	10	87.9 (5.0)	84.4 (5.1)
	5	90.8 (2.9)	94.4 (3.2)
	1	82.8 (5.6)	85.8 (4.3)
	0.5	83.1 (8.1)	93.1 (9.6)
FHG	10	87.8 (4.2)	81.2 (2.1)
	5	98.4 (4.8)	83.8 (7.1)
	1	91.8 (4.3)	88.1 (3.6)
	0.5	99.9 (5.5)	100.3 (6.2)
MMF	10	96.0 (0.9)	88.0 (1.4)
	5	90.9 (2.7)	80.8 (1.1)
	1	98.7 (3.5)	95.1 (2.3)
	0.5	83.3 (9.5)	86.5 (9.2)

RN Radix Notoginseng
FHG Fructus Hordei Germinatus
MMF Massa Medicata Fermentata

was performed by pushing the plunger into the tube until the bottom of the clean-up column was reached, and then the column eluted with 2 × 4 mL of acetonitrile:water (80:20, *v*/*v*). The eluate was collected and combined. Subsequently, the eluate was evaporated to dryness in a rotary vacuum evaporator at 60 °C. Afterwards the residue was resolved in 1.0 mL of mobile phase (water–acetonitrile–methanol, 90:5:5, *v*/*v*/*v*) and 50 µL was injected into the LC system.

Results and Discussion

Optimization of Extraction Solvents

The first step in the analysis of trichothecenes involves an efficient extraction of the toxin from a complex matrix. The most used extraction solvents for DON and NIV were aqueous acetonitrile (61.4%), or aqueous methanol (17.5%) [22]. Five different ratios of aqueous acetonitrile and aqueous methanol were studied through the analysis of black Radix Notoginseng samples spiked with DON and NIV at a level of 5.0 mg kg^{-1}. The results are shown in Table 2. Each experiment was replicated three times and the relative standard deviations (RSD) were 0.8–3.6%. The results showed that acetonitrile–water (80:20, *v*/*v*) was the best solvent for the extraction of DON and NIV from the various TCMs analyzed in this study.

Optimization of Clean-Up Columns

The clean-up methods for DON and NIV in TCM tested in this work yielded various different recoveries depending on the types of clean-up columns. The results are shown in Table 2. Each experiment was replicated three times and the relative standard deviations (RSD) were 1.2–4.7%. Through comparison of five different clean-up columns (Mycosep225, Bond Elut Mycotoxin, Puri Tox^{SR} TC-M160, Puri Tox^{SR} TC-T220 and Puri Tox^{SR} TC-T200 DON purification column), the Puri Tox^{SR} TC-T200 DON purification column was selected as best for the clean-up of DON and NIV from the various TCMs analyzed in this study. Previously, Mycosep225 column was usually used for the clean-up of trichothecenes from grains [13]. Bond Elut Mycotoxin column, a new SPE sorbent, was applied for the clean-up of *Fusarium* toxin from contaminated cereals and cereal-based foods. PuriTox^{SR} TC-T200 purification column, which contains a mixture of reversed phase, ion exclusion, and ion exchange adsorbents, was formulated and designed to provide optimum sample extract purification prior to the analyses of mycotoxins by LC, GC and TLC methodology. This packing material retains interferences such as protein compounds, fats, carbohydrates, and pigments that are extracted from food and feed products by organic solvents. The mycotoxins are not retained by the packing materials and flow through the column. The present results showed that good recoveries and purification effect for DON and NIV were obtained with the Puri Tox^{SR} TC-T200 DON purification column.

Method Validation

The calibration curves were constructed by plotting the peak areas versus the concentrations of DON and NIV. Satisfactory calibration curves of DON and NIV were obtained. The results showed good linearity in the range of 0.2–20 µg mL^{-1} of DON and NIV ($Y = 61{,}734X - 3{,}240$ for DON, and $Y = 65{,}424X + 3{,}288$ for NIV, $r = 0.9999$ for both DON and NIV). The limits of detection of the method were 63 µg kg^{-1} for DON and 50.0 µg kg^{-1} for NIV based on a signal-to-noise ratio of 3:1. The limits of quantification of the method were 125.0 µg kg^{-1} for DON and 100.0 µg kg^{-1} for NIV, based on a signal-to-noise ratio of 10:1.

The precision of the method was tested by measuring both intra-day and inter-day reproducibility from analysis of standard solutions. The intra-day reproducibility of the assay was determined by six consecutive injections each of 0.5 µg DON and NIV between 10 a.m. and 4 p.m., within the same day. Peak area RSD was 1.49% for DON and 1.65% for NIV. The inter-day variability of the assay was determined by five consecutive injections each of 0.5 µg DON and NIV between 9 and 11 a.m. on five different days. Peak area RSD was 1.29% for DON and 1.24% for NIV.

The stability of the standard solution was determined by monitoring the peak area responses over a period of 12 h, from 9 a.m. to 21 p.m., within the same day. The results showed that the peak areas of DON and NIV remained almost unchanged with RSD 1.23% for DON and RSD 1.20% for NIV.

The accuracy of the method was confirmed by the following recovery tests. Three different TCMs, Radix Notoginseng, Fructus Hordei Germinatus and Massa Medicata Fermentata,

were selected for the recovery test. They represented TCMs of different origins and no interfering peaks occurred in their chromatograms. The samples were spiked with four different amounts of DON and NIV before extraction. The spiked samples were extracted and analyzed under the conditions as described above (Fig. 1). Overall average recoveries for three different TCMs spiked at levels of 0.5, 1, 5, and 10 mg kg^{-1} ranged from 82.8 to 99.9% for DON with RSDs between 0.9 and 9.5%, 80.8 to 100.3% for NIV with RSDs between 1.1 and 9.6%, as shown in Table 3. The recovery results demonstrated that this method can be regarded as accurate.

Application to Samples

The method reported here was utilized for the simultaneous determination of DON and NIV in TCMs purchased from the market with different origins ($n = 30$). As shown in Fig. 1, even if the impurity peaks were present in the chromatogram, they did not interfere with determination of DON and NIV in the samples. From Table 1, the results showed that none of these contained DON and NIV above the limits of detection.

Conclusions

In summary, an economical and efficient LC-UV method has been developed for the simultaneous determination of DON and NIV in TCM with a DON purification column clean-up for the first time. The method has good recovery and reproducibility. No peak interfering with DON and NIV was observed. In comparison with previously reported methods [23], the present method has the advantage of low detection limits in complex matrixes. The method is suitable for the simultaneous determination of DON and NIV in TCMs with different origins and can be used for monitoring DON and NIV levels in TCMs in order to evaluate its potential hazard to human health.

Acknowledgments

This work was supported by the Important New Drug Research Project of the Ministry of Science and Technology of China (2009ZX09502-025) and Scientific Research Project of Traditional Chinese Medicine Vocation in 2008 (200807042).

References

1. Kotal F, Holadova K, Hajslova J, Poustka J, Radova Z (1999) J Chromatogr A 830:219–225. doi:10.1016/S0021-9673(98)00720-1
2. Sudakin DL (2003) Toxicol Lett 143:97–107. doi:10.1016/S0378-4274(03)00116-4
3. Eriksen GS, Pettersson H (2004) Anim Feed Sci Technol 114:205–239. doi:10.1016/j.anifeedsci.2003.08.008
4. Pestka JJ (2007) Anim Feed Sci Technol 137:283–298. doi:10.1016/j.anifeedsci.2007.06.006
5. Miller JD (1995) J Stored Prod Res 31:1–16. doi:10.1016/0022-474X(94)00039-V
6. Milanez TV, Valente-Soares LM, Baptista GG (2006) Food Control 17:293–298. doi:10.1006/j.foodcont.2004.11.002
7. Tutelyan VA (2004) Toxicol Lett 153:173–179. doi:10.1016/j.toxlet.2004.04.042
8. Schollenberger M, Muller HM, Rufle M, Drochner W (2008) Food Control 19:475–482. doi:10.1016/j.foodcont.2007.05.012
9. Commission Regulation 856/2005 (2005) Off J Commun L 143:3–8
10. Sokolovic M, Simpraga B (2006) Food Control 17:733–740. doi:10.1016/j.foodcont.2005.05.001
11. Krska R, Baumgartner S, Josephs R (2001) Fresenius J Anal Chem 371:285–299. doi:10.1007/s002160100992
12. Papadopoulou-Bouraoui A, Vrabcheva T, Valzacchi S, Stroka J, Anklam E (2004) Food Addit Contam 21:607–617. doi:10.1080/02652030410001677745
13. Radova Z, Holadova K, Hajslova J (1998) J Chromatogr A 829:259–267. doi:10.1016/S0021-9673(98)00868-1
14. Jestoi M, Ritieni A, Rizzo A (2004) J Agric Food Chem 52:1464–1469. doi:10.1021/jf035130g
15. Schollenberger M, Lauber U, Terry-Jara H, Suchy S, Drochner W, Muller HM (1998) J Chromatogr A 815:123–132. doi:10.1016/S0021-9673(98)00454-3
16. Omurtag GZ, Beyoglu D (2007) Food Control 18:163–166. doi:10.1016/j.foodcont.2005.09.007
17. Jajic I, Juric V, Abramovic B (2008) Food Control 19:545–550. doi:10.1016/j.foodcont.2007.05.009
18. Razzazi-Fazeli E, Bohm J, Luf W (1999) J Chromatogr A 854:45–55. doi:10.1016/S0021-9673(99)00616-0
19. Dall'Asta C, Sforza S, Galaverna G, Dossena A, Marchelli R (2004) J Chromatogr A 1054:389–395. doi:10.1016/j.chroma.2004.07.081
20. Santos L, Sonia M, Sanchis V, Ramos AJ (2009) J Sci Food Agric 89:1802–1807. doi:10.1002/jsfa.3647
21. Yue YT, Zhang XF, Pan JY, Ou-Yang Z, Wu J, Yang MH (2010) Chromatographia 71:533–538. doi:10.1365/s10337-010-1477-1
22. Samar MM, Resnik S (2002) Food Sci Technol Int 8:257–268. doi:10.1106/108201302029023
23. Cahill LS, Kruger SC, McAlice BT, Ramsey CS, Prioli R, Kohn B (1999) J Chromatogr A 859:23–28. doi:10.1016/S0021-9673(99)00846-8