ASPEC® 274: Automated Extraction of Deoxynivalenol (DON) from Wheat

APPLICATION NOTE 1012

APPLICATION BENEFITS

Deoxynivalenol (DON) is a mycotoxin that occurs predominantly in grains such as wheat, barley, oats, rye, and maize. The consumption of food contaminated with DON is associated with outbreaks of gastroenteritis in humans. Automated methods are well-suited for quantification of toxins because they are accurate, reduce the time required for laboratory analysis, and reduce human errors.

SOLUTIONS

Sample pre-treatment was automated using a Gilson ASPEC® 274 and extracts were analyzed by liquid chromatography. Validation parameters such as accuracy, precision, limits of detection, and quantification were evaluated by the analysis of reference material B-MYC0856, (naturally contaminated wheat). Automated control of volumes and flow rates using the ASPEC 274 enabled precise and reproducible recovery of DON.

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INTRODUCTION

Wheat is one of the main cereals cultivated in the world and its flour is widely used in the food industry. Wheat and its products can be affected by several microorganisms, especially toxigenic fungi, capable of producing mycotoxins.[1] Determination of Deoxynivalenol (DON) levels in these products is critical for public health.

DON (3α, 7α, 15-trihydroxy-12, 13-epoxytrichothec-9-en-8-one) (Figure 1) is a mycotoxin belonging to the trichothecene family produced by fungi of the genus *Fusarium*. Also known as vomitoxin, it occurs mainly in cereals, such as wheat and rice. DON is stable at high temperatures and can therefore survive processing to contaminate wheat-containing products. Indeed, several acute disease outbreaks related to DON contamination have been reported in Asia. Ingestion of food contaminated with DON is associated with the following symptoms in humans: nausea, vomiting, vertigo, gastrointestinal disturbances, and diarrhea. In addition, *in vitro* studies have demonstrated that DON has genotoxic potential.[1]

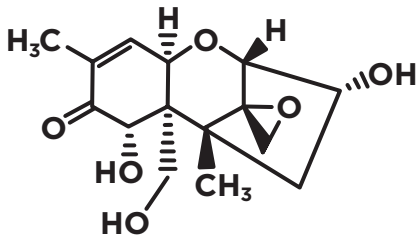

Figure 1
Chemical Structure of Deoxynivalenol (DON), CAS nº 51481-10-8

On 8 February 2017 the maximum tolerable limits of deoxynivalenol were established by Brazilian legislation RDC No.138, in some products including wheat, whole wheat flour, pasta, biscuit, and others. The maximum limit for whole wheat and whole wheat flour is 1250 µg/kg.[2]

The determination of DON in food comprises the following steps: extraction, cleanup, elution, and quantification by chromatography. In this work, the ASPEC® 274 System was used in the automation of the cleanup and elution of the analyte. The validation of analytical methods ensures the reliability of the analytical results. Reference materials should be used whenever possible, and are essential for evaluation accuracy.[3] The wheat reference material B-MYC0856 was used to assess the accuracy, precision (repeatability and intermediate precision), the limits of detection, and quantification.

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MATERIALS AND METHODS

Chemicals and Reagents

Chromatography-grade methanol and acetonitrile were obtained from Merck, Darmstadt, Germany. DON (D0156) was obtained from Sigma Aldrich, St. Louis, MO, USA. Naturally contaminated wheat flour with a DON certified value of 877 µg/kg ± 23 µg/kg was obtained from LGC Standards (Wesel, Germany) (B-MYC0856).

Instrumentation

Automated sample preparation, and subsequent separation and detection, were carried out using the following instrumentation:

- ASPEC® 274, an automated solid phase extraction system, which comprises a liquid handler and two dual syringe pumps.
  (Gilson, Inc.; Middleton, WI, USA)
- Alliance 2695 HPLC System, which comprises a pump, autosampler, degasser, oven, and diode array detector (Model 2475).
  (Waters Corporation; Milford, MA, USA)
- Horizon Shaker KS501
  (IKA Labortechnik; Staufen, Germany)
- Centrifuge NT 812
  (Nova Técnica; Piracicaba, Brazil)
- Milli-Q water purification and filtration system.
  (Millipore; Bedford, MA, USA)
- Concentrator 5301
  (Eppendorf; Hamburg, Germany)

Columns

- DonStar™ R immunoaffinity cartridge
  (PN COIAC5004). (Romer Labs; Union, MO, USA)
- C18 reverse phase analytical column
  (PN R0086200C5), Microsorb-MV 100-5,
  250 x 4.6 mm, 5 µm.
  (Varian Medical Systems; Walnut Creek, CA)
- C18 reverse phase guard column, ZORBAX,
  4 x 3 mm.
  (Agilent Technologies; Palo Alto, CA, USA)

Method

100 mL of deionized water with resistivity of 18 MΩ.cm was added to 12.5 g of the sample and the mixture was shaken on a horizontal shaker table for 30 min at 200 rpm. The sample was then transferred to polypropylene (PP) centrifuge tubes and centrifuged at 3,000 rpm for 10 minutes. The supernatant was filtered through microfiber paper. The filtrate was collected in 10 mL test tubes and transferred to the ASPEC 274 System for cleanup. DonStar™ R immunoaffinity cartridges were pre-sealed with Gilson 3 mL sealing caps. Sample preparation on the ASPEC 274 System was divided into two steps under Gilson TRILUTION® LH Software control: cleanup and elution (Figure 2).

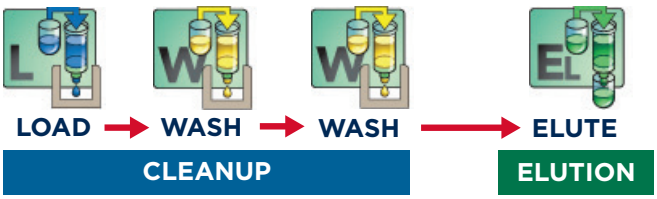

Figure 2
Steps in the TRILUTION® LH Software: Cleanup of the Extract and Elution of DON

Summary of the Automated Sample Preparation

Step 1: Cleanup

- Load 2 mL of the filtered aqueous extract onto the cartridges at a flow rate of 0.5 mL/min.
- Wash cartridges with 2 x 10 mL of ultrapure water at a flow rate of 2 mL/min.
- Dry by centrifugation in the concentrator at 1,400 rpm for 5 min. Because of the low pressure of the nitrogen gas in the laboratory, the cartridges were removed from the Gilson system.

Step 2: Elution

- Reload the cartridges in the Gilson system. Elute DON with 2 mL of methanol at a flow rate of 0.5 mL/min. Collect the eluate in 5 mL test tubes.

The test tubes were withdrawn from the system and the solvent was evaporated to dryness in a water bath at 45°C under N2 flow. The dried extract was dissolved in 1.0 mL of water, by vortexing, and filtered through a 0.45 µm PTFE membrane. The filtrate was collected for further liquid chromatographic analysis. DON quantification was performed by an external standardization method.

Chromatographic Conditions

- Mobile phase: water:methanol:acetonitrile (80:15:5; v/v/v)
- Flow rate: 0.7 mL/min
- Column temperature: 30°C
- Injection volume: 50 µL
- Detection: ultraviolet at 220 nm

RESULTS AND DISCUSSION

We report the automation of a sample preparation method for isolation of DON from wheat samples. Cleanup and elution steps were automated using a ASPEC 274 System. DON determination was done using an HPLC method. The chromatogram resulting from the analysis of a wheat sample shows that matrix background removal was efficient when using the immunoaffinity cartridge for the cleanup (Figure 3). The validation parameters of accuracy, precision, limits of detection and quantification were evaluated as suggested by the National Institute of Metrology, Standardization and Industrial Quality.[3]

The limits of detection and quantification were estimated from three to 10 times the signal-to-noise ratio (S/N), respectively (Table 1). The limit of quantification matches the first point of the calibration curve (Figure 4).

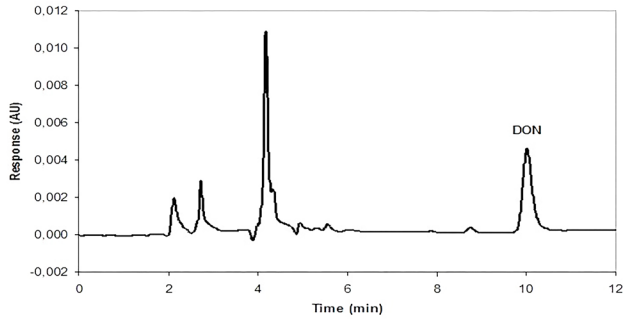

Figure 3
Chromatogram obtained by HPLC-UV in the determination of DON in a wheat sample (λ = 220 nm, DON retention time: 10.1 min.)

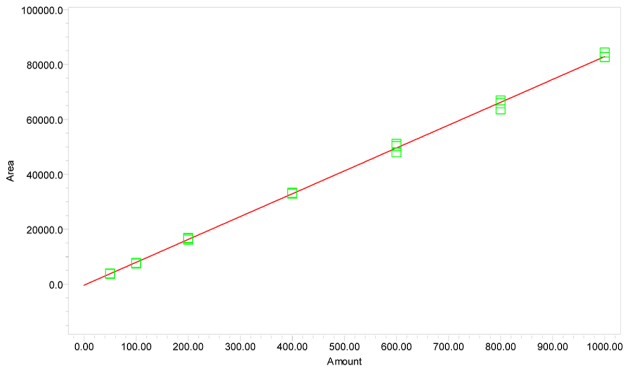

Figure 4
Calibration curve (the concentration range of 50–1000 ng/mL corresponds to a concentration of 200–4000 µg/kg in the final sample)

Table 1:
Limits of Detection and Quantification for DON

LIMIT	DON (µg/kg)
Detection	60.0
Quantification	200.0

Table 2
Recovery of DON

SAMPLE	AVERAGE (n=6) (µg/kg)	SD* (µg/kg)	CV** (%)	CERTIFICATED VALUE (µg/kg)	AVERAGE RECOVERY (%)
Reference Material	894.4	21.1	2.4	877 ± 23	102.0

*Standard Deviation (SD) **Coefficient of Variation (CV)

Table 3
DON Concentration Determined in the Reference Material for Evaluation of Precision

DAY	ANALYST	DON AVERAGE CONCENTRATION (n=6) (µg/kg)	SD* (µg/kg)	CV** (%)	CERTIFICATED VALUE (µg/kg)
1	1	894.4[a]	21.1	2.4	877 ± 23
	2	886.8[a]	20.6	2.3	
2	1	882.3[a]	24.7	2.8	
	2	892.5[a]	23.9	2.7	

*Standard Deviation (SD) **Coefficient of Variation (CV) [a]Means do not have significant difference by the Tukey test (p>0.05)

The accuracy of the method was evaluated through recovery tests. The reference material was analyzed in six replicates at a single concentration level. The average concentration of DON, standard deviation and coefficient of variation were calculated. The percent recovery ranged from 99.5 to 104.9%, that is, an average recovery of 102.0%, which indicates good accuracy of the method (Table 2).

Precision represents the dispersion of results obtained by independent and repeated measurements of the same sample, under defined conditions. It is usually expressed numerically by the standard deviation or the coefficient of variation.[4] The analytical conditions for the evaluation of the method precision included the same measurement procedure, the same analyst, and the same instrument under the same instrumental parameters. Six analyses of the reference material were performed on the same day. The average of the concentrations determined for DON, the standard deviation and coefficient of variation are presented in Table 3. These data indicate a good repeatability of the method.

The intermediate precision was evaluated by six analyses of the same sample, performed by two analysts on two different days. The coefficient of variations on the same and different days were below 3% (Table 3). Results were submitted to the analysis of variance which indicated no significant variation between results obtained on different days by different analysts in the same laboratory.

CONCLUSIONS AND BENEFITS

Automation of the extraction of DON in wheat enables accurate control of volumes and flow rate. This ensures consistent results day-to-day with coefficients of variation lower than 3%. The accuracy of the method was demonstrated by the average recovery of 102%. The method is in accordance with current Brazilian legislation.

A manual step can be easily incorporated into the automated process to comply with lab requirements. TRILUTION LH can combine sequential and batch protocols in the same application for improved reproducibility.

The ASPEC 274 is able to process four samples in parallel and increases efficiency and sample throughput.

REFERENCES

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ACKNOWLEDGMENTS

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ORDERING INFORMATION

PART NUMBER	DESCRIPTION	QUANTITY
2614010	ASPEC® 274	1