# Application of a simple and rapid pre-treatment procedure in the high performance liquid chromatographic analysis of deoxynivalenol and zearalenone in beer

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**Summary**

In this study, newly developed analytical methods for determination of deoxynivalenol (DON) and zearalenone (ZEA) in beer are described. Four processes for beer pre-treatment prior to HPLC analysis of both mycotoxins using diode-array (for DON), and fluorescent detection (for ZEA) were examined. Easy-to-use procedures of beer refining using specific immunoaffinity columns (IAC) Donprep and Easi-Extract® Zearalenone (both from R-Biopharm Rhône, Glasgow, United Kingdom) were designed for DON and ZEA isolation, respectively. Maximum capacities of both tested IAC were explored in order to minimize the risk of their overloading with the beer sample. The minimal detectable amount was 6 $\mu$g.l$^{-1}$ for DON and 4 $\mu$g.l$^{-1}$ for ZEA. In a random survey, fourteen commercial beer samples were investigated for DON and ZEA by these methods. While ZEA was not detected in any tested sample, trace concentrations of DON (6–7 $\mu$g.l$^{-1}$) were found in 29% of the analysed beer samples. Concentrations higher than 7 $\mu$g.l$^{-1}$ were detected in 28% of beer samples. The highest concentration of DON detected by this method in a lager beer was 33.2 $\mu$g.l$^{-1}$.

**Keywords**

deoxynivalenol; zearalenone; beer; immunoaffinity column; high performance liquid chromatography (HPLC)

Deoxynivalenol (DON) and zearalenone (ZEA) are major secondary metabolites produced by fungi of the *Fusarium* genus, which are abundant in cereals such as maize, wheat, barley, oats and rye as well as in processed grains such as bread, malt and beer. They cause scab or head blight on cereals [1, 2]. The malignant DON symptoms include vomiting, nausea, diarrhea and damage of blood cells. Although ZEA exhibits only low acute toxicity, estrogenic effects have been reported in some species for it and for some of its metabolites, in particular α- and β-zearalenol [3]. DON is a type B trichothecene toxin whose creation is closely associated with *F. graminearum* and *F. culmorum*, the soil fungi that are important plant pathogens grown on the crop in the field [2]. Zearalenone occurs in cereal grains in a simultaneous presence of the other *Fusarium* toxins, including trichothecenes and fumonisins [4]. In some cases, also the presence of zearalenone derivatives (e.g. α-zearalenol, β-zearalenol, α-zearalanol, β-zearalanol) was detected [5]. Toxic effect of *Fusarium* toxins on humans and animals is well-proven and documented in numerous studies [3, 6–10]. Based on recent data on DON and ZEA toxicity, the Scientific Committee on Food (SCF) has established for DON a tolerable daily intake (TDI) of 1 $\mu$g.kg$^{-1}$ of body weight, and for ZEA a temporary TDI of 0.2 $\mu$g.kg$^{-1}$ of body weight [11]. However, the data were available only for the European region, so that more accurate information from other parts of the world will be needed.

Mycotoxins originating in barley and malt contaminated with *Fusarium* may have a serious impact on the food production, mainly in the malting and brewing industry. The published data on natural occurrence of DON in barley malt as well as ZEA in barley malt and corn indicate that these toxins enter into brewing process as contaminants of the malting barley and other grains used in beer production. As follows from the recently published data, ZEA content is of lower interest in comparison with DON. Somewhat controversial data were published on the concentrations of DON in barley and malt. In one study, the final concentration of DON in malt was approximately the same or lower

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than its concentration estimated in barley [12], while in the second study, *Fusarium* mycotoxins including DON were present in malt in higher concentrations in comparison to the original barley [13]. Due to its high temperature resistance, DON can persist in the mash and get into the final beer [12]. Regarding the ZEA content, this toxin exhibited a moderate stability during a fermentation step [14] and most of it was converted by brewing strains *Saccharomyces cerevisiae* into its derivatives α-zearalenol and β-zearalenol [15]. Contamination of barley and malt with *Fusarium* is also responsible for the so-called gushing in the packaged beer, which is defined as an uncontrolled and spontaneous over-foaming after opening a bottle or can [16].

In general, quality requirements for malting barley are reasonably strict. *Fusarium* head blight (FHB) and the presence of DON in barley directly impacts the quality of both malt and beer. Barley from areas with conditions susceptible to FHB occurrence is routinely screened for DON. Barley with DON concentrations over 500 $\mu g.kg^{-1}$ is normally not used in the malting process [17]. As for ZEA, concentrations lower than 50 $\mu g.kg^{-1}$ of barley are acceptable [18]. Concerning the consumption data of mycotoxins from beer, the dietary daily intake of ZEA was up to 0.3 $ng.kg^{-1}$ of body weight among consumers in the United Kingdom [11].

Various concentrations of DON and ZEA in beers and grains intended to be brewed were reported [19-26] (Tab. 1). The published data vary in a broad interval, in dependence on some key parameters associated with climatic, agricultural and brewing conditions.

As far as it is known, the results of determination of mycotoxins strongly depend on the method used and may differ between methods. The quantitative and qualitative procedures for trichothecenes determination were reviewed [27, 28]. In general, the key steps necessary for the pretreatment and the determination of trichothecenes and ZEA are extraction and clean-up of the extracts, followed by the detection and quantification of the toxins.

Liquid–liquid partitioning, solid-phase extraction (SPE), column chromatography, application of immunoaffinity columns (IAC) or multifunctional clean-up columns are the most frequently used methods for sample preparation. Various combinations of solvents were used in the liquid-liquid partitioning for the extraction of the trichothecenes, including methanol and acetonitrile in water, or ethyl acetate and acetonitrile at various ratios [29]. SPE, or eventually column chromatography, usually involve variant stationary phases such as silica gel, florisil, aluminium oxide, charcoal and C8 or C18 reversed phases, corresponding to the required polarity range of the adsorbent. Modified charcoal-alumina-based columns (Mycosep; Romer Labs, Union, Missouri, USA) were reported to give very good results [30]. Recently, IAC for isolation of individual mycotoxins became commercially available, e.g. DONtest HPLC and ZearalaTest (Vicam Science Technology, Watertown, Massachusetts, USA), Donprep and Easi-Extract Zearalenone (R-Biopharm Rhône, Glasgow, United Kingdom) [31]. These columns can be applied only for a single toxin, what is their significant limitation.

The widely applied screening quantitative method based on the enzyme-linked immunosorbent

**Tab. 1.** Occurrence of deoxynivalenol and zearalenone in beers and adjuncts used in brewery, according to recent publications.

| Commodity | Deoxynivalenol | Zearalenone | Reference |
|---|---|---|---|
| Maize for beer brewing | data not published | 100-800 $\mu g.kg^{-1}$ | [19] |
| Corn malt | data not published | up to 4000 $\mu g.kg^{-1}$ | [19] |
| Barley and malt | data not published | 100-200 $\mu g.kg^{-1}$ | [20] |
| Beer | data not published | 90-4600 $\mu g.l^{-1}$ | [19] |
| | data not published | 245-1320 $\mu g.l^{-1}$ | [14] |
| | 7-70 $\mu g.l^{-1}$ | data not published | [21] |
| | data not published | 2.6-426 $\mu g.l^{-1}$ | [22] |
| | 0.3-50.3 $\mu g.l^{-1}$ | data not published | [23] |
| | 4-56.7 $\mu g.l^{-1}$ | data not published | [24] |
| | 5-20 $\mu g.l^{-1}$ | data not published | [25] |
| Beer conventional<br>Beer organic | 6-22.1 $\mu g.l^{-1}$<br>6-14.0 $\mu g.l^{-1}$ | data not published<br>data not published | [26] |

assay (ELISA) is used particularly for the simultaneous determination of several trichothecenes [21, 24] as well as of ZEA [32]. ELISA is a rapid and simple method but it may suffer from low sensitivity because of its cross-reactivity with deoxynivalenol-related compounds [33]. However, some studies on beer indicated very low detection limits (LOD) estimated by ELISA, e.g. 6 $\mu$g [21] or 2 $\mu$g [31] of DON per litre of beer.

The chromatographic methods such as thin layer chromatography (TLC) [22, 34], high performance liquid chromatography (HPLC) [31] and gas chromatography (GC) [35] combined with different detection systems are the common reference methods used for the confirmation of positive screening methods in the analysis of mycotoxins.

In this paper, four beer pre-treatment procedures were investigated and compared in order to recover a simple, precise, and rapid liquid-chromatographic method for DON and ZEA determination in beer, applying specific IAC for cleaning-up of each toxin. A technique of high performance liquid chromatography (HPLC) with ultraviolet (UV) and fluorescent (FL) detection was used for DON and ZEA identification, respectively. The methods were internally validated in accordance with EURACHEM [36] and IUPAC [37] guidance principles. In addition, incidence of both DON and ZEA in a limited random panel of commercial beers produced in Slovakia was examined by the developed methods.

## MATERIALS AND METHODS

### Chemicals

Crystalline standard of deoxynivalenol (> 97% purity) and zearalenone (98% purity) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). HPLC-grade methanol and acetonitrile Chromasolv were obtained from Sigma-Aldrich Laborchemikalien (Seelze, Germany). Glacial acetic acid 99.6% p. a. was provided by AFT (Bratislava, Slovakia). Potassium dihydrogen phosphate, sodium chloride, potassium chloride and -hydrochloric acid (35%), all p. a., were purchased from Lachema (Brno, Czech Republic). Disodium hydrogen phosphate was from Merck (Darmstadt, Germany). Deionized water used as a constituent of mobile phases was prepared in Analyst HP (Purite, Oxon, United Kingdom).

### Standard solutions

The stock standard solutions of DON in acetonitrile and ZEA in methanol were prepared at a concentration of 1 mg.ml$^{-1}$ and were kept at –18 °C. The working (calibration) standards of DON were prepared by the dilution of the stock solution in the mobile phase (acetonitrile : deionized water, 10 : 90, v/v). The working (calibration) standards of ZEA were prepared by the dilution of the stock solution in methanol.

### Phosphate buffered saline

The phosphate buffered saline (PBS) solution was used to dilute beer samples or beer sample extracts immediately before IAC clean-up in ZEA analysis. It was prepared as follows: 8.0 g of sodium chloride, 1.2 g of disodium hydrogen phosphate, 0.2 g of potassium dihydrogen phosphate and 0.2 g of potassium chloride were dissolved in approximately 990 ml of deionized water and the pH value was adjusted to 7.4 with 35% hydrochloric acid. The solution was filled with deionized water up to the volume of one litre.

### Beer samples

Fourteen different bottled samples of domestic beers (11 of lager type, 3 of dark beers) with alcohol contents ranging from 3.1% to 5.0%, v/v) were obtained at a local retail. The samples were selected to cover all beer production localities in Slovakia. For research purposes, one batch of lager (the alcohol content of 4.3%, v/v, the average pH value of 4.8) was degassed in an ultrasonic bath and subsequently filtered through a paper filter. Beer samples were stored at 6 °C.

### Sample preparation

Just before an immunoaffinity clean-up process, four procedures for beer pre-treatment were examined. With this intention, one litre of beer was contaminated with the stock standard solution to a single concentration of 250 $\mu$g.l$^{-1}$ for both DON and ZEA.

#### Procedure 1

A volume of 10 ml of the beer sample was diluted in 10 ml of acetonitrile (pH value of the final solution was 5.4). The solution was shaken on an orbital shaker for 5 min and then filtered through a paper filter. An aliquot of 10 ml or 2 ml of the filtrate was applied to IAC for DON and ZEA determination, respectively.

#### Procedure 2

An identical sequence as described in the first procedure was used till the filtration point. From the filtrate obtained, an aliquot volume of 10 ml (for DON) or 2 ml (for ZEA) was evaporated to dryness on a rotary evaporator at a water bath

temperature of 50 °C. The dried residue was then dissolved in 5 ml of the mixture of acetonitrile : deionized water, 10 : 90, v/v (for DON, pH value of the mixture was 6.8) or in 5 ml of the mixture of acetonitrile : PBS, 20 : 80, v/v (for ZEA, pH value of the mixture was 8.1). The entire volumes were then applied to IAC.

Procedure 3

A beer sample was subjected to centrifugation at 704 *g* for 10 min. An aliquot of 5 ml (for DON) or 1 ml (for ZEA) of the supernatant was applied to IAC.

Procedure 4

A volume of 5 ml (for DON) or 1 ml (for ZEA) of the degassed and filtered beer sample was directly applied to IAC.

**Clean-up on immunoaffinity columns**

IAC Donprep and Easi-Extract Zearalenone (R-Biopharm Rhône, Glasgow, United Kingdom) were employed for DON and ZEA isolation, respectively. At first, the columns were conditioned with the filling solution being present at each IAC. Then, a specific volume of the beer sample, as described at pre-treatment procedures, was passed through the column by gravity or by slight vacuum, if appropriate. The column was washed with 10 ml (for DON) or 20 ml (for ZEA) of deionized water and the washing eluates were discarded. After drying the column under vacuum for 10 min, DON or ZEA were eluted using 2 ml of methanol or with 1.5 ml of acetonitrile and 1.5 ml of water, respectively. Elution solvents were subsequently evaporated on a rotary evaporator at a water bath temperature of 50 °C. The obtained residue was dissolved in 0.25 ml of a mixture of acetonitrile : deionized water, 10 : 90, v/v (for DON) or in 0.25 ml of the mixture of acetonitrile : deionized water, 60 : 40, v/v (for ZEA).

**HPLC procedures**

Analyses were performed on a HPLC system of Agilent Technologies 1100 Series (Waldbronn, Germany), equipped with an auto-sampler and the analytical column Zorbax SB-C18, 4.6 × 250 mm i.d. with the sorbent particle size of 5 $\mu$m, connected to a guard column Zorbax SB-C18, 12.5 × 4.6 mm i.d., with the sorbent particle size of 5 $\mu$m (both from Agilent Technologies). A diode-array detector (DAD) was used for DON analyses, set at following wavelengths: sample/bandwidth - 220/16 nm, reference/bandwidth - 360/80 nm. A mixture of acetonitrile : deionized water, 10 : 90, v/v, was used as the mobile phase at a flow rate of 1 ml·min$^{-1}$. Involving the auto-sampler, the injected volume of the sample was 50 $\mu$l.

For ZEA analyses, a fluorescence detector (FLD) was employed at excitation and emission wavelengths of 274 nm and 440 nm, respectively,. The excitation range from 220 nm to 380 nm and the emission from 300 nm to 500 nm were used to obtain fluorescence spectra. A mixture of acetonitrile and acidified water (12 ml of glacial acetic acid in one litre of deionized water), 60 : 40, v/v, at a flow rate of 0.5 ml.min$^{-1}$, was used as the mobile phase. Injection of 20 $\mu$l of the sample was performed by auto-sampler. The column temperature was adjusted to 25 °C, automatically controlled by Agilent ChemStation (Agilent Technologies).

**Identification of mycotoxins**

Since an equilibrium isocratic elution was used for both DON and ZEA analyses, identification of the tested mycotoxins was, in the first step, accomplished on the basis of retention times and, in the second step, on the basis of spectra at specific wavelength ranges. The peak purity was checked as well when confirmation of identity of mycotoxins was essential.

**Validation of the methods**

The methods for DON and ZEA determination in beer were validated by means of calibration and estimation of the range of linearity, precision (expressed as internal repeatability), accuracy (recovery tests) and expanded uncertainty of the measurement. Values of the detection limit (*LOD*) and limit of quantification (*LOQ*) were also determined for both DON and ZEA..

The calibration measurements were carried out with series of DON and ZEA standard solutions. Individual calibration curves were created using the Excel XP software (Microsoft, Redmont, Washington, USA). Responses of DAD and FLD were linear in the range of concentrations of 7–250 $\mu$g.l$^{-1}$ for DON, and 7.4–416 $\mu$g.l$^{-1}$ for ZEA, respectively. LOD and LOQ values were calculated as signal-to-noise ratio S/N = 3 and S/N = 10, respectively.

The recovery of DON and ZEA was studied with beer samples that were not naturally contaminated but spiked with standard solutions of mycotoxins. The recovery rates of individual mycotoxins were calculated using the following equation:

$$R\,[\%] = (C_{spiked\ sample} / C_{\text{spiking solution}}) \times 100 \quad (1)$$

where $C$ represents concentration in $\mu$g·l$^{-1}$.

The uncertainty of measurement was evaluated as combined uncertainty $U_c$ with the covering factor of 2 and a 95% confidence interval. The major

sources taken into consideration in $U_c$ estimation were the values of relative standard deviation of the repeatability ($RSD_r$), dilution of the standard solution and the beer sample, and the calibration curve linearity.

The final concentration of both mycotoxins in beer was calculated using the equation:

$$C\ [\mu g \cdot l^{-1}] = C^* \cdot F \quad (2)$$

where $C^*$ is the concentration of the mycotoxin estimated from the calibration curve in $\mu g \cdot ml^{-1}$ (injection concentration), $F$ is the conversion factor which included the initial and final volumes of the sample taken into analysis as well as the factor 1000 which represents the concentration in $\mu g \cdot l^{-1}$.

The entire analyses of the beer samples were carried out in duplicate, from which the standard deviation (*SD*) and the relative standard deviation (*RSD*) were calculated.

## RESULTS AND DISCUSSION

Deoxynivalenol and zearalenone differ in chemical structure, thus their unambiguous HPLC determination may require a sophisticated approach. DON is more polar than ZEA as its molecule contains an unsaturated keto-function. This group absorbs UV radiation of short wavelengths in the region of 218–222 nm, so UV-detection may be utilized for its identification. Acetonitrile and mixtures of acetonitrile and water were suggested to be the most proper solvents in DON analysis [38]. ZEA as a weakly acidic substance is usually extracted from solid matrices by basic-acid liquid-liquid extraction using harmful organic solvents such as chloroform and methylene chloride [39, 40]. In some recently applied ZEA extraction techniques, the health hazardous solvents were replaced by various mixtures of acetonitrile, methanol and water [41–43].

Beer is easy to analyse because the distribution of mycotoxins is supposed to be homogenous in this matrix. This is why we intended to simplify the pre-treatment procedures as much as possible. The experiments were performed in duplicate with beer artificially contaminated with either individual DON and ZEA solutions or by the addition of their mixture (250 $\mu g \cdot l^{-1}$ each). The effectiveness of the sample preparation was assessed on the basis of determination of recovery rates of mycotoxins, as presented in Tab. 2.

Acetonitrile and water were mostly used as extraction, dilution and elution solvents. For ZEA detection, PBS in combination with acetonitrile was applied. As follows from the our results, procedure 1 in which acetonitrile was employed as a dilution agent, was ineffective for both mycotoxins. Recoveries higher than 50% were achieved for DON by procedure 2, but these were taken as still insufficient. High recovery rates of above 100% were achieved for ZEA by this method, which were probably influenced by the addition of the mixture of acetonitrile : PBS, 20 : 80 v/v, at pH adjustment prior to IAC cleaning process. Procedures 3 and 4 gained practically identical results for both DON and ZEA and based on these results, centrifugation of beer was omitted in further work. Procedure 4 had good recovery rates for DON (90% on average) and for ZEA (103% on average), when the mycotoxins were not present in the beer together.

The precision of the pre-treatment methods estimated as the relative standard deviation

**Tab. 2.** Efficacy of different beer pre-treatment procedures illustrated as recovery rates of mycotoxins.

| Procedure | Mycotoxin present in beer | Average concentration determined [$\mu g.l^{-1}$] | *SD* [$\mu g.l^{-1}$] | *RSD* [%] | Average recovery rate [%] |
|---|---|---|---|---|---|
| 1 | DON | 4.6 | 1.1 | 23.9 | 2 |
| | DON and ZEA | 5.6 and 29.6 | 0.4 and 2.7 | 7.1 and 9.1 | 2 and 10 |
| | ZEA | 48.9 | 10.2 | 20.9 | 17 |
| 2 | DON | 145.7 | 30.7 | 21.1 | 58 |
| | DON and ZEA | 155.7 and 320.5 | 13.7 and 87.8 | 8.8 and 27.4 | 62 and 126 |
| | ZEA | 310.7 | 79.0 | 25.4 | 122 |
| 3, 4 | DON | 224.7 | 11.4 | 5.1 | 90 |
| | DON and ZEA | 186.8 and 335.7 | 4.7 and 26.6 | 2.5 and 7.3 | 75 and 116 |
| | ZEA | 296.5 | 1.4 | 0.4 | 103 |

Added amount of DON and ZEA in beer was 250 $\mu g \cdot l^{-1}$, $n = 2$.
*SD* – standard deviation, *RSD* – relative standard deviation.

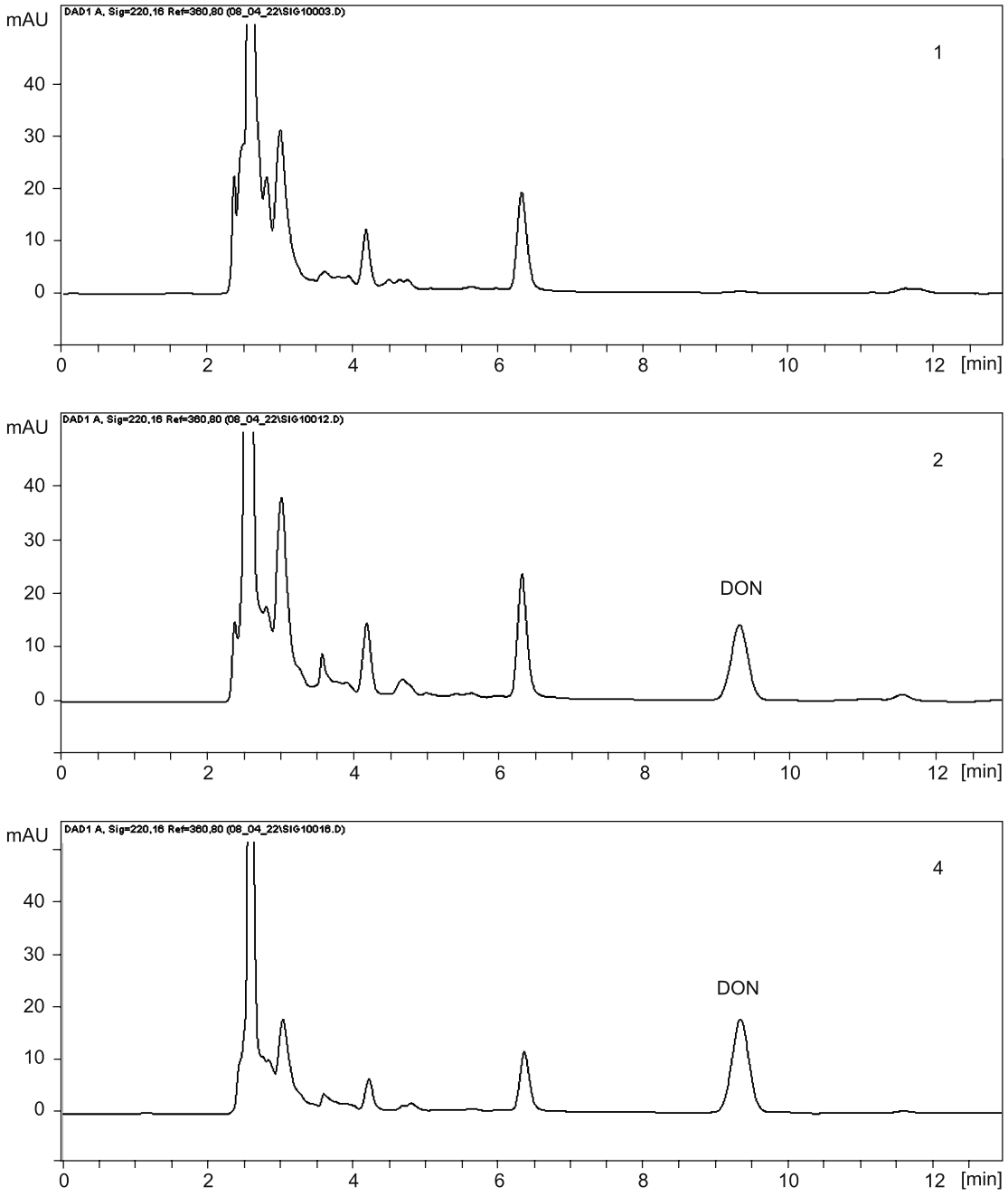


**Fig. 1.** Typical chromatogram of DON extracted from the spiked beer sample using pre-treatment procedures 1, 2 and 4, respectively.

(*RSD*) was very low for procedures 1 and 2 (*RSD* > 20%). On the other hand, significantly higher precision (*RSD* 0.4–7.3%) for both mycotoxins was determined for procedure 4. When using procedure 4, the lowest background appeared in chromatograms (Fig. 1). Since direct application of beer samples onto IAC was used in this procedure, additional measurements were carried out to test the impact of the beer matrix on the analysis, as well as the maximum IAC capacity for both mycotoxins. A series of standard solutions of both mycotoxins and a beer sample spiked with the standard solution of mycotoxins to the concentration levels of 5, 25, 80, 250, and 500 $\mu g \cdot l^{-1}$ were analysed by HPLC, directly after the immunoaffinity clean-up using the specific IAC. Each concentration level

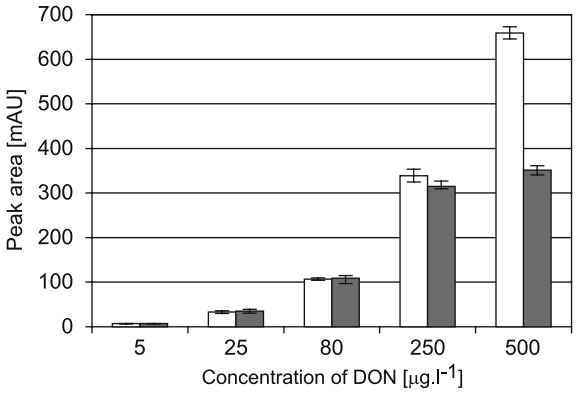


**Fig. 2.** The analytical responses of DON in dependence on its concentration.
White columns – standard solutions of DON, grey columns – beer samples spiked with standard solutions of DON. All solutions and beer samples were cleaned up on an immunoaffinity column Donprep before HPLC analysis.

was analysed in duplicate. As shown in Fig. 2, the maximum capacity of the Donprep column was 250 $\mu g \cdot l^{-1}$ (i.e., 1.25 $\mu$g DON in 5 ml of the beer sample). The highest binding capacity of Easi-Extract Zearalenone column for ZEA was 420 $\mu g \cdot l^{-1}$ (i.e., 0.42 $\mu$g ZEA in 1 ml of the beer sample; data not shown). Overloading of IAC above the mentioned concentration levels of both mycotoxins resulted in stable analytical responses.

As follows from the evaluation of the results obtained, procedure 4 represents a method of choice for the validation process of both DON and ZEA. The effect of the matrix on the analytical separation process was minimal when this method was used. In addition, the advantage of this method in comparison to others was that no special sample pre-treatment was required, except for the immunoaffinity clean-up of the beer sample.

The validation parameters for both methods are in details summarized in Tab. 3. The *LOD* values achieved are in good agreement with recently published data for analysis of beers by HPLC or gas chromatography, ranging from 5 $\mu g \cdot l^{-1}$ to 25 $\mu g \cdot l^{-1}$ for DON and from 1 $\mu g \cdot l^{-1}$ to 5 $\mu g \cdot l^{-1}$ for ZEA [44].

The recovery rates of mycotoxins were determined at three (DON) and two (ZEA) concentration levels, covering the range from 17 $\mu g \cdot l^{-1}$ to 250 $\mu g \cdot l^{-1}$. The recovery rates differed only non-significantly from those estimated for beer pre-treatment at the level of 250 $\mu g \cdot l^{-1}$. The precision of the analytical methods, expressed as internal repeatability, was verified by tenfold analyses of spiked matrix samples. The investigated concentrations were 27 $\mu g \cdot l^{-1}$ and 250 $\mu g \cdot l^{-1}$ for DON, and 17 $\mu g \cdot l^{-1}$ and 245 $\mu g \cdot l^{-1}$ for ZEA. $RSD_r$ values reached a maximum of 11.4% for DON and 9.5% for ZEA (Tab. 3).

The chromatograms of both DON and ZEA extracted from the spiked and non-spiked beer samples were very transparent, without any matrix interferences near the retention time of the mycotoxins (Fig. 3, Fig. 4).

**Tab. 3**. Validation parameters of the HPLC methods for determination of DON and ZEA in beer.

| Parameter | DON | | ZEA | |
|---|---|---|---|---|
| *LOD* [$\mu g.l^{-1}$] | 6 | | 4 | |
| *LOQ* [$\mu g.l^{-1}$] | 7 | | 7.4 | |
| Linearity range [$\mu g.l^{-1}$] | 7–250 | | 7.4–250 | |
| Precision $RSD_r$ [%] | 11.4–2.6 | | 9.5–4.2 | |
| Expanded uncertainty $2U_c$ [%] | 23–17 | | 17–7 | |
| Linearity correlation factor $R^2$ | 0.9999 | | 0.9995 | |
| Slope | 0.0147 | | 0.0014 | |
| Intercept | 0.010 | | –0.007 | |
| Conversion factor *F* | 50 | | 250 | |
| Accuracy | Contamination level [$\mu g \cdot l^{-1}$] | Recovery rate [%] | Contamination level [$\mu g \cdot l^{-1}$] | Recovery rate [%] |
| | 27 | 105 | 17 | 85 |
| | 80 | 99 | 245 | 90 |
| | 250 | 100 | | |

*LOD* - limit of detection, *LOQ* - limit of quantification, $RSD_r$ – relative standard deviation of repeatability, $2U_c$ – combined uncertainty of the measurement with the covering factor of 2.

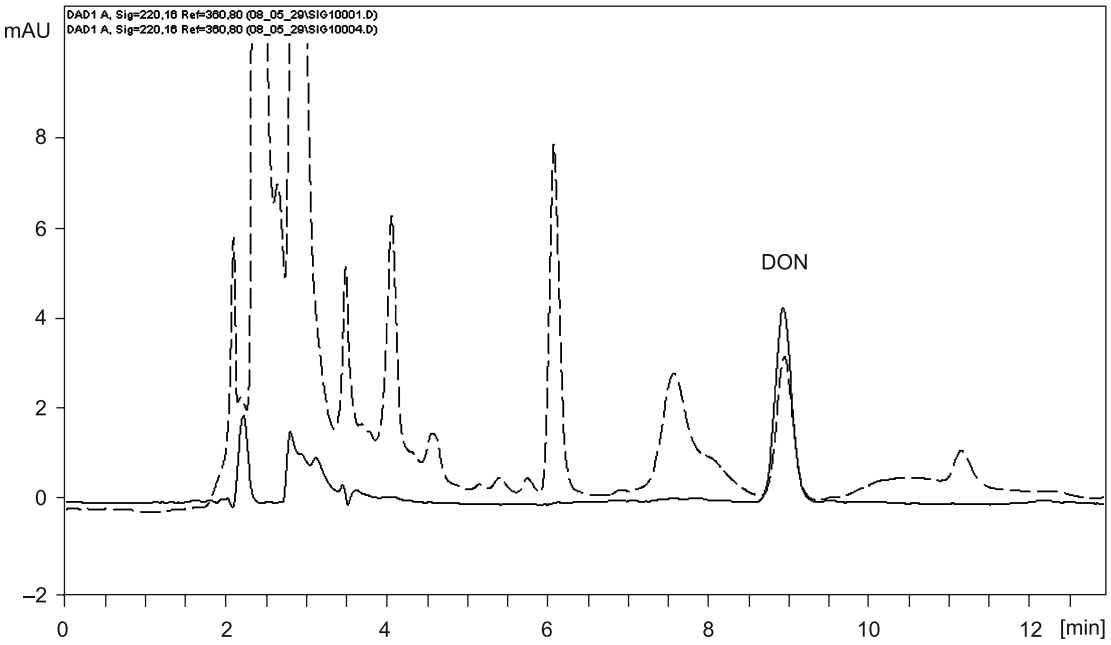


**Fig. 3.** Typical chromatogram of DON in the standard solution.
Injection concentration of 1 $\mu$g.ml$^{-1}$ – continual line, extract of naturally contaminated beer – dashed line.

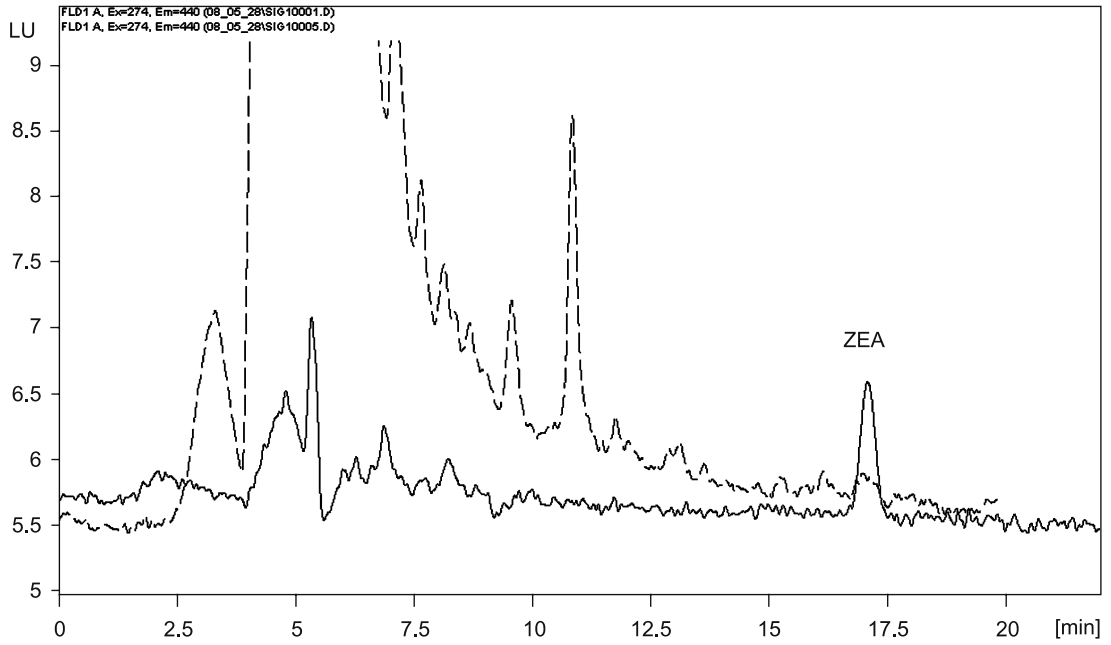


**Fig. 4.** Typical chromatogram of ZEA in the standard solution.
Injection concentration of 0.01 $\mu$g.ml$^{-1}$ – continual line, extract of non-contaminated beer – dashed line.

**Tab. 4.** Contamination of beers produced in Slovakia with DON.

| | Number of beer samples | Concentration of DON in beer samples [$\mu$g·l$^{-1}$] |
|---|---|---|
| Total number of samples | 14 (100%) | – |
| Non-contaminated samples | 6 (43%) | < 6 |
| Samples with DON traces | 4 (29%) | 6–7 |
| Positive samples | 4 (28%) | 7.6–33.2 |

The described validated methods were used for determination of DON and ZEA in beers produced in Slovakia. In total, 14 beer samples were tested, involving both lager and dark beers filled in glass bottles. Results obtained revealed that no beer sample under the test contained ZEA, but DON presence in beers was evident (Tab. 4). A maximum concentration of 33.2 $\mu g.l^{-1}$ of DON in lager type beer was detected. Standard deviations in positively tested beer samples varied in the interval of 0.7–7.9 $\mu g.l^{-1}$. DON was also present in all dark beers investigated at trace concentrations in between *LOD* and *LOQ* (from 6 $\mu g.l^{-1}$ to 7 $\mu g.l^{-1}$). The fact that no ZEA was found in the tested beers illustrates its rare presence in beers compared to DON. High amounts of ZEA were detected e.g. in African locally brewed beers [21], while no significant levels of ZEA were found in beer produced in Europe.

## CONCLUSIONS

Rapid, simple, sensitive and easy-to-use methods of DON and ZEA determination in beer are presented. The proposed pre-treatment enables direct isolation of the mycotoxins from beer samples using specific immunoaffinity columns (Donprep and Easi-Extract Zearalenone, respectively). In addition, results obtained reveal that overloading of these columns regarding the matrix or regarding mycotoxins is eliminated. The analytical separation is rapid, taking a maximum of 20 min of experimental time. The methods represent an innovation in monitoring of mycotoxin contamination of beers in laboratories equipped with a standard HPLC apparatus. Moreover, they have an advantage of requiring no extensive sample preparation before the analysis.

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