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RESEARCH PAPER

Molecularly imprinted vs. reversed-phase extraction for the determination of zearalenone: a method development and critical comparison of sample clean-up efficiency achieved in an on-line coupled SPE chromatography system

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Abstract

Sample preparation prior to chromatographic separation plays an important role in the analytical process. To avoid time-consuming and manual handling sample-prep, automated on-line techniques such as on-line SPE-HPLC are therefore preferred. In this study, two different on-line extraction approaches for mycotoxin/endocrine disruptor zearalenone (ZEA) determination using either molecularly imprinted polymer (MIP) with selective cavities and binding sites for extraction or a reversed-phase sorbent C18 providing non-selective interactions have been developed, validated, and compared. The validation characteristics were compared and the two methods were evaluated as being almost equal in terms of linearity, repeatability, precision, and recovery. Recoveries were in the range of 99.0–100.1% and limits of detection were found the same for both methods (1.5 μg L^{-1}). Method precision calculated for spiked beer samples was better for C18 sorbent (2.5 vs. 5.4% RSD). No significant differences in the selectivity of either extraction method were observed. The possible reasons and further details associated with this finding are discussed. Finally, both validated methods were applied for the determination of ZEA contamination in beer samples. Due to ZEA's native fluorescence, chromatographic separation with fluorimetric detection (λ_{ex} = 270 nm and $\lambda_{em,}$ = 458 nm) was selected.

Keywords Molecularly imprinted polymers · On-line extraction · On-line SPE chromatography · Solid-phase extraction · Mycotoxin · Zearalenone

Introduction

Endocrine disruptors are chemical compounds that interfere with the endocrine system, bind to hormone receptors, activate them, and act as a competitive substrate for them and for metabolism enzymes or enzymes involved in synthesis [1]. A great public interest is observed in the light of the increasing trend in the incidence of hormone-related diseases like breast cancer and reproductive problems in the human population [2]. In the case of estrogen receptors, various natural and xenobiotic substances including phytoestrogens (isoflavones, coumestans, and lignans) and xenoestrogens (polychlorinated biphenyls, bisphenol A, and nonylphenols) are able to bind to the receptor [3]. Zearalenone (ZEA, also known as F-2 toxin), a mycotoxin produced by molds of the *Fusarium* genera, also shows estrogen-like activity. Despite its non-steroidal structure, ZEA and its metabolites compete with endogenous steroids, activate estrogen receptors, and disrupt steroid synthesis and metabolism [1, 3, 4]. Its hydroxylated metabolite α-zearalenol has even higher estrogenic potency [1, 3]. Moreover, in concentrations higher than those with a hormonal effect, zearalenone has been described as genotoxic [5], immunotoxic [6, 7], and is being investigated for possible carcinogenicity in high doses.

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Human exposure to zearalenone occurs mostly via grains and cereals. Although mycoestrogen ZEA affects animals as well, the carry-over into meat and milk is not a serious risk [1]. Since ZEA is heat-stable and remains almost unchanged after heating up to 125 °C [8], it is also present in cereal products and it is a known fact that ZEA, among other mycotoxins like ochratoxin A and deoxynivalenol, may also be transferred in beer [9]. Although serious contaminations were reported even in levels up to 400 μg kg^{-1} in African beers [10], mostly there were determined concentrations less than 2 μg kg^{-1} [11, 12] or under limit of detection [13]. Current European legislation sets a maximum permitted limit of zearalenone in foods and feeds [14]. However, no specific limit for beer exists. The lowest permitted concentration is set for baby food at 20 μg kg^{-1}.

A crucial problem with analyzing mycotoxins is their determination at very low and trace level concentrations [15] in complex matrices requiring sensitive detection techniques and proper clean-up or selective extraction procedures. Zearalenone is a rather lipophilic compound (log P 4.37) with the strongest acidic character pKa 8.54 (ChemAxon). Its natural fluorescent properties enable sensitive fluorimetric detection. However, beer contains additional naturally fluorescent compounds such as amino acids, B vitamins, and phenolic compounds. Hence, sample preparation including selective extraction and matrix interference clean-up is an important step before the analysis is performed.

For zearalenone determination, several electrochemical methods have been described [16, 17], in advance involving electrochemical immunosensors [18, 19]. Immunochemical methods are also often used [11, 20]. Nevertheless, the most common technique is high-performance liquid chromatography with selective and sensitive fluorescence detection [21–25], and less often with UV detection [25]. Tandem mass spectrometry (MS) detection [26–28], which often enables the simultaneous determination of several mycotoxins, has also been widely used [29–33].

Pre-extraction and clean-up step is usually needed prior to chromatographic separation. In addition, non-selective and insufficient sample clean-up yields co-eluting matrix compounds and/or enhanced or suppressed ionization in MS detection [26]. Simple liquid extraction from a solid sample [25] is usually improved by solid-phase extraction using for example C18 sorbents [17, 26], or even multistep sequential solid-phase extraction (SPE) clean-up [34]. Some specifically designed sorbents have been used for the selective extraction of ZEA [23, 35]. Nowadays, QuEChERS is the preferred clean-up approach because it is universal, simple, and suitable for multi-analyte determination [24, 32, 33, 36]. There is a boom of micro-modifications being added to traditional extraction techniques such as dispersive liquid-liquid microextraction (dLLME) [21, 37], dispersive suspended microextraction [28], and hollow-fiber liquid-phase microextraction followed by a GC-MS/MS analysis [38].

For selective extraction are also available several immuno-affinity columns (IAC) [29, 30, 33, 39]. However, their coupling to on-line SPE chromatography system is limited due to their specific extraction conditions and low possibility of reusing. The IAC consist of peptidic structure of antibodies which are not so stable mechanically and chemically. The problematic point of IAC coupling with chromatography is the need for specific extraction steps based on immunoafinity interactions which are less compatible with following chromatographic separation. Moreover, their extraction capacity is limited and sorbents are expensive. Therefore, selective sorbents based on molecularly imprinted polymers (MIP) are considered to be very promising and able to replace IAC due to their higher capacity, stability, and lower cost [15, 40]. They can be home-made or commercially available products can be used [22, 27, 41–44]. The automation of extraction along with miniaturization and increase in selectivity are the current most promising trends in extraction techniques. Several approaches for on-line extraction coupled with chromatographic determination using a column-switching system have been published including those involving a covalent SPE home-made sorbent [23], TurboFlow™ column [45], and IAC [33].

In this work, we chose a molecularly imprinted polymer specific for zearalenone in the extraction step. The selectivity of molecularly imprinted solid-phase extraction (MISPE), in comparison with immunoaffinity sorbents, has already been proven [22]. However, the on-line coupling of MISPE to an HPLC system is not widespread due to its difficult optimization and tuning the compatibility between the extraction and separation step [46, 47]. This is especially true since the extraction on MIP is a highly optimized and specified procedure to achieve selective interactions with polymer; otherwise, the imprinting is pointless. The MISPE procedure usually requires more washing steps, a drying sub-step, the use of specific solvents to achieve specific interactions with target analyte, and a lack of compatibility, e.g., for on-line coupling to chromatography systems, which is often a problem. On the other hand, MIPs are ideal for on-line connection due to their chemical stability, high extraction capacity, and selectivity. Therefore, the aim of this work was development of a new method for the selective determination of zearalenone using on-line MISPE-HPLC. For comparison, a newly developed on-line SPE-HPLC method using the C18 extraction sorbent was evaluated and its selectivity compared to determine that it is possible to achieve desired results with both on-line extraction techniques. Making a critical evaluation of the advantages and disadvantages of both approaches, and using both validated methods for real sample analyses was the second aim of our study. To the best of our knowledge, no method for ZEA mycotoxin determination using on-line MISPE-HPLC-FLD has been published yet.

Materials and methods

Chemicals and materials

Standard zearalenone, glacial acetic acid, and organic solvents of HPLC gradient grade (acetonitrile and methanol) were purchased from Sigma-Aldrich (Czech Republic). Ultra-pure water was obtained from a Milli-Q purification system (Millipore, USA).

Commercial zearalenone-selective SPE columns packed with an MIP sorbent, AFFINIMIP® SPE Zearalenone, were obtained from Polyintell (Affinisep, France). Approximately 80 mg of MIP sorbent was filled into a stainless steel 10 × 4.6 mm cartridge and used for the on-line connection with the chromatography system.

An Ascentis Express C18, 5 × 4.6 mm, 5 μm particle size core-shell guard column for comparative extraction method was obtained from Sigma-Aldrich (Czech Republic). A Kinetex C18, 150 × 4.6 mm, 5 μm particle size analytical core-shell column was purchased from Phenomenex (USA).

Thirty bottled beer samples were bought in a local supermarket, and each sample was pipetted into a glass vial immediately after opening the bottle. The unfiltered beers containing yeast suspension were filtered through a 0.45 μm PTFE filter prior to their analysis.

Equipment and the chromatographic system

The Shimadzu Prominence HPLC system (Shimadzu Corporation, Japan) consisted of an SIL-20AC autosampler, three LC-20AD solvent delivery modules with a DGU-AS online degasser, a CTO-20AC column oven with an FCV-12AH high-pressure six-port switching valve, a RF-10A XL fluorescence detector, and a CBM-20A communication module. The system control, data acquisition, and data evaluation were performed by the Shimadzu "LC Lab-Solution" software (Shimadzu Corporation, Japan).

Preparation of standard solutions

A standard stock solution of ZEA (100 mg L^{-1}) was prepared by dissolving the substance in methanol and stored at −20 °C. Working solutions for the optimization and validation of the method were obtained by diluting the stock solution with water to a concentration of 1 mg L^{-1}, which was further diluted with water or blank beer for the preparation of calibration solutions. Working solutions were kept in a refrigerator.

On-line extraction and separation using MISPE-HPLC

On-line extraction was performed directly in a chromatographic system using a column-switching mode. Two columns, an MIP extraction precolumn and an analytical column for the separation, were connected via a six-port switching valve. Switching between two positions, the extraction and separation mode was chosen. In the first position, a 50-μL sample was injected in the MIP extraction column in a washing mobile phase consisting of acetonitrile and 2% aqueous acetic acid (10/90, *v*/*v*). This solution was chosen according to the MIP producer's recommendation to keep the clean-up step selectivity as high as possible. Polar interferences from the matrix were washed out while the analyte was retained. After 2 min, the valve was switched and the mobile phase eluted retained zearalenone from the extraction column onto the Kinetex C18 analytical column where the separation was carried out using gradient elution. The initial mobile phase was acetonitrile-water (35/65, *v*/*v*), kept for 1.5 min followed by a linear increase of gradient up to 100% acetonitrile within 5.5 min. Both columns were washed with acetonitrile for 1 min, then the acetonitrile percentage was decreased to the initial 35%, and the valve was switched to the first position to let the columns return to their initial conditions. The total analysis time, including the on-line MISPE step and both column equilibrations, lasted for 13.0 min.

The fluorescence detector parameters were set at a ZEA maximum fluorescence wavelengths of 270 and 458 nm for the excitation and emission wavelengths, respectively.

On-line SPE-HPLC using a C18 extraction column for selectivity comparison

Using the same column-switching system described in the previous section, the C18 guard column replaced the MIP for extraction to make a comparison. The online SPE-HPLC was performed under the same conditions with the same gradient as the one used for the MISPE-HPLC method except for the washing mobile phase that was changed to methanol–2% aqueous acetic acid (40/60, *v*/*v*) because ZEA was retained on the C18 in the washing mobile phase containing up to 40% methanol.

Results and discussion

Optimization of on-line MISPE-HPLC

The composition of the washing mobile phase was tested for MIP extraction by increasing the percentage of the organic solvent, acetonitrile or methanol. Using a higher fraction of organic solvent in the washing mobile phase enabled a more efficient clean-up of the matrix. However, the analyte washing out from the MIP sorbent was observed at concentrations of the organic phase exceeding 10% acetonitrile and 15% methanol. The effect of the percentage of the organic phase on ZEA retention in the MIP sorbent is shown in Fig. 1a. Since ZEA is a weak acid, an acidified washing mobile phase was tested to

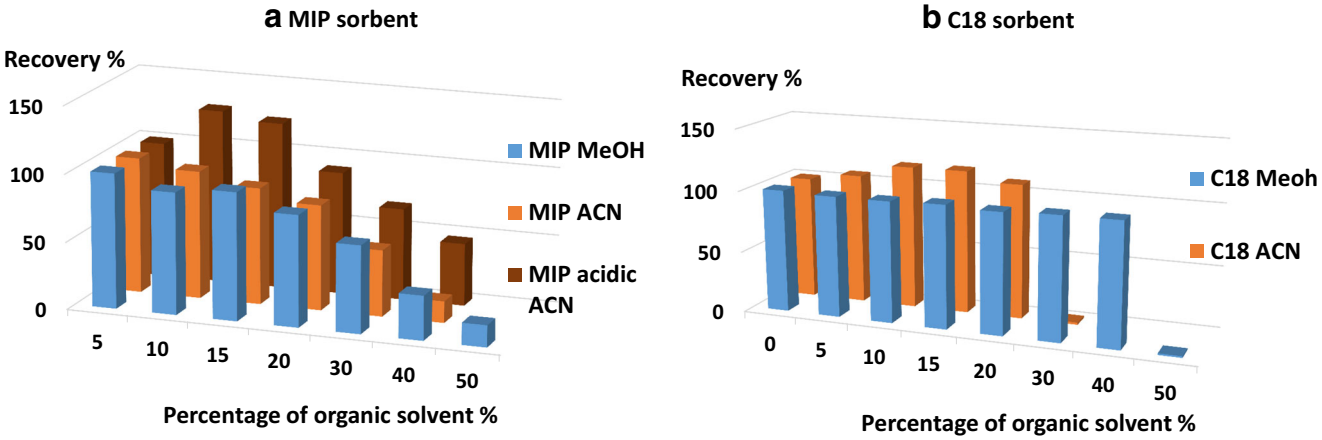

Fig. 1 Retention of ZEA on extraction sorbent depending on percentage of organic solvent and pH of washing mobile phase. **a** MIP sorbent. **b** C18 sorbent

avoid ionization of the molecule. As can be observed in Fig. 2, acidifying the washing mobile phase significantly improved matrix clean-up and the fluorescence signal of ZEA was slightly enhanced (leading to higher recovery values for acidified ACN in Fig. 1a). However, the analyte was not retained any stronger. Thus, 10% acetonitrile was set as the maximum concentration of the organic component in 2% aqueous acetic acid. Figure 2 confirms that almost the same chromatogram demonstrating interference removal was obtained with acidified 15% methanol. The off-line SPE protocol recommended by the MIP producer requires washing with an acidified acetonitrile solution. Therefore, this recommendation was preferred during the method optimization. Washing for longer than 2 min was found worthless because no significant removal of the matrix interferences was observed.

One of the most critical steps of on-line SPE-HPLC optimization is the choice of the elution solvent used to achieve elution from the extraction column and transfer to the analytical column at the same time. The eluate is transferred directly in the analytical column where first the analyte retention is needed followed by the separation. Off-line elution from MIP SPE is usually performed using 100% organic solvent. However, this percentage is not compatible with on-line chromatography separation because of the column overloading effect, undesirable peak tailing or fronting, and low separation efficiency. The off-line MIP protocol recommendation is to evaporate the solvent until dry to remove the residues of the washing solution followed by elution with 2 mL 98% acidified methanol. In our case, it was necessary to omit this step in the on-line column-switching system. Therefore, the optimized elution solvent gradually eluted ZEA from MIP, starting with just 35% acetonitrile solution so as not to overload the analytical column and to enable separation. Figure 1 shows that no retention of ZEA on the MIP sorbent occurs after acetonitrile percentage exceeds 40%. Thus, the linear gradient of the mobile phase eluted ZEA as a narrow symmetric peak. When subsequently increasing the gradient up to 100% acetonitrile, a complete elution and equilibration of MIP was achieved. This approach enabled that the separation was carried out in the gradient, and the columns were finally washed free of any remaining interferences.

Some steps of the MISPE were implemented using the instruction sheet provided by the MIP producer for off-

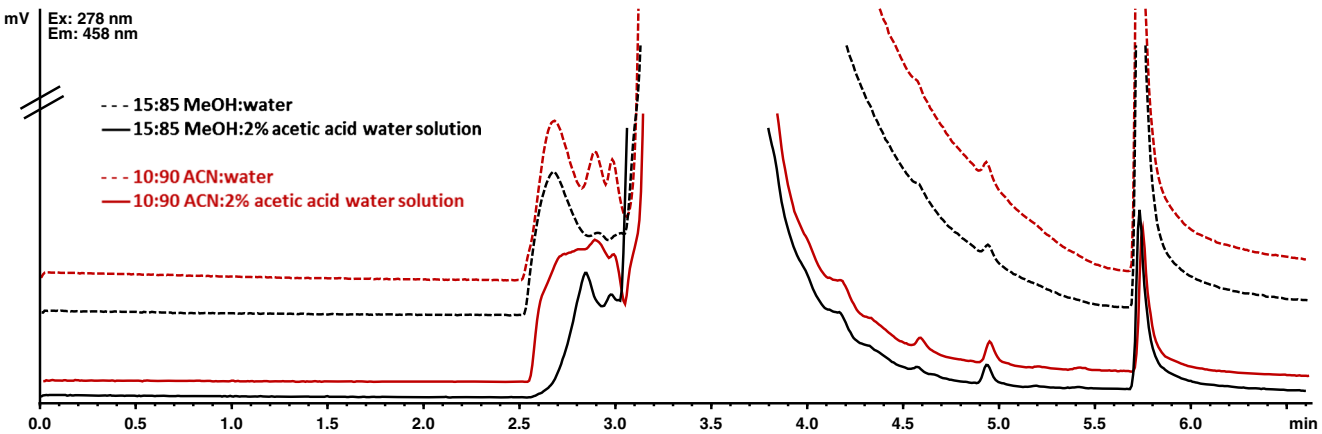

Fig. 2 Matrix clean-up using different washing solvents on MIP; the influence of acidifying effect (red lines: ACN, black lines: MeOH, dashed lines: not adjusted, full lines: acidified with acetic acid)

line work including slower loading the sample for proper interaction with the MIP. The flow rate was only 0.5 mL min^{-1} during the sample injection in MIP while a flow rate of 1 mL min^{-1} was used for the remaining steps of the analysis. Omitting the sorbent drying substep was possible since the eluate was not evaporated and reconstituted at the end. These steps were not conducted in our on-line column-switching system where the eluate in the mobile phase continues directly in the analytical column. Acetic acid in the washing mobile phase was used and the separation was modulated to a retention time of ZEA of about 8 min to achieve optimal resolution from residual matrix components.

The choice of the separation column was made according to the analyzed matrix. Beer contains a number of naturally fluorescent compounds, mostly more hydrophilic than ZEA. Thus, the gradient had to be optimized to first elute the mass of ballast components and then ZEA. A shorter separation time did not enable sufficient resolution between ZEA and residual interferences from the beer samples. The universal C18 reversed stationary phase exhibited good selectivity. A column length of 15 cm instead of 10 cm was used to reach a better resolution of ZEA from the matrix components and the gradient length was extended. The on-line MISPE-HPLC chromatogram of blank beer and ZEA spiked beer under optimal extraction and separation conditions is presented in Fig. 3a.

Optimization of on-line C18 SPE-HPLC

The on-line (C18)-SPE-HPLC method was developed as a comparative method for determining which approach is better in terms of selectivity in the on-line mode, simplicity of the method development, and optimization. From this point of view, the validation parameters for both methods were also evaluated and compared. Gradient design, detection parameters, flow rates, and injection volumes used were the same as with the MISPE method. Only the washing mobile phase has to be re-optimized due to the different extraction mechanism. Like MIP, increasing percentage of organic solvent in the washing mobile phase was tested for matrix clean-up and analyte retention (shown in Fig. 1b). A methanol concentration of 40% in acidified aqueous solution still provided good retention and recovery of ZEA.

Since ZEA is a rather lipophilic mycotoxin with log P 4.37 (partition coefficient), it was strongly retained on the C18 extraction column. As the washing mobile phase could contain 40% methanol, the matrix ballast was removed more efficiently than from the MIP sorbent with just 10% acetonitrile. A comparison of the clean-up efficiency using both sorbents at the upper limit of organic solvents in the washing mobile phase is presented in Fig. 4. It is obvious that the peak of the interferences was washed out more efficiently using the extraction based on hydrophobic interactions using C18 reversed phase. Figure 3b shows the on-line (C18)-SPE-HPLC

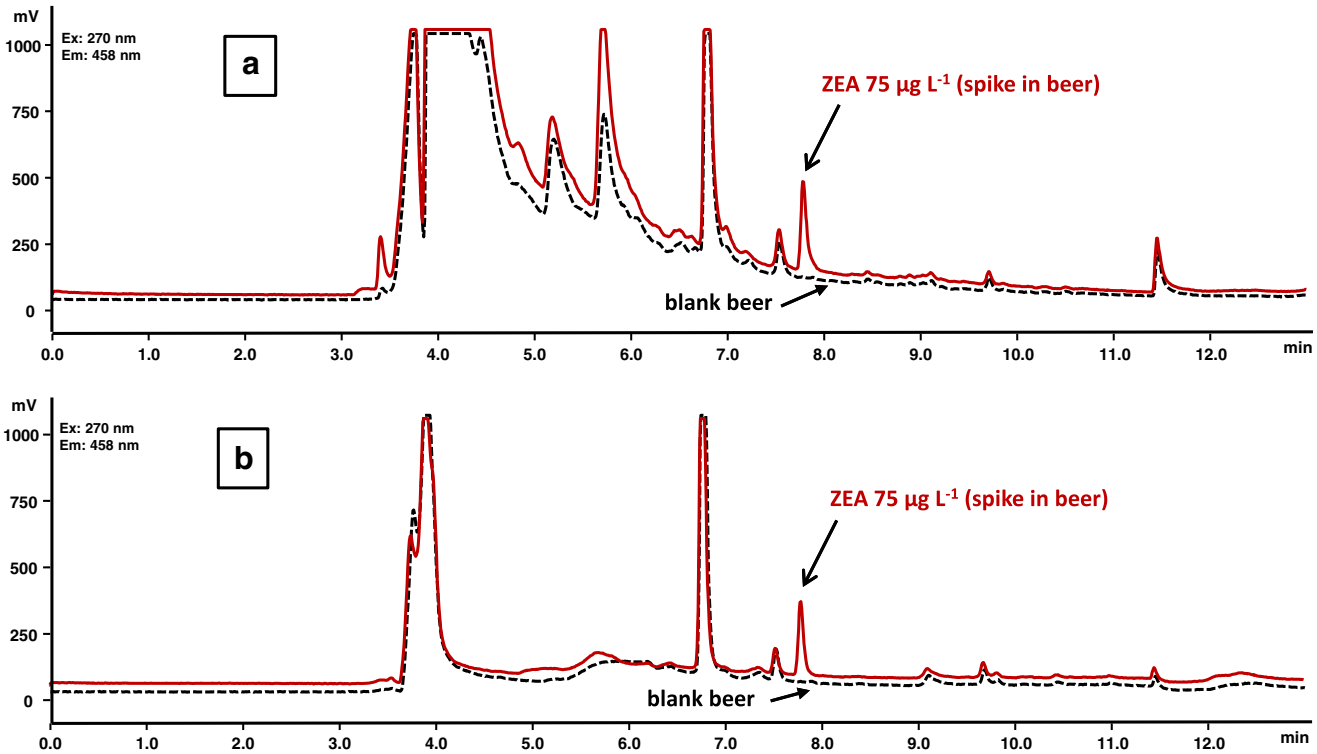

Fig. 3 Chromatograms of blank beer and spiked beer sample under the optimal condition of extraction and separation obtained by on-line MISPE-HPLC method (**a**) and C18-SPE-HPLC method (**b**)

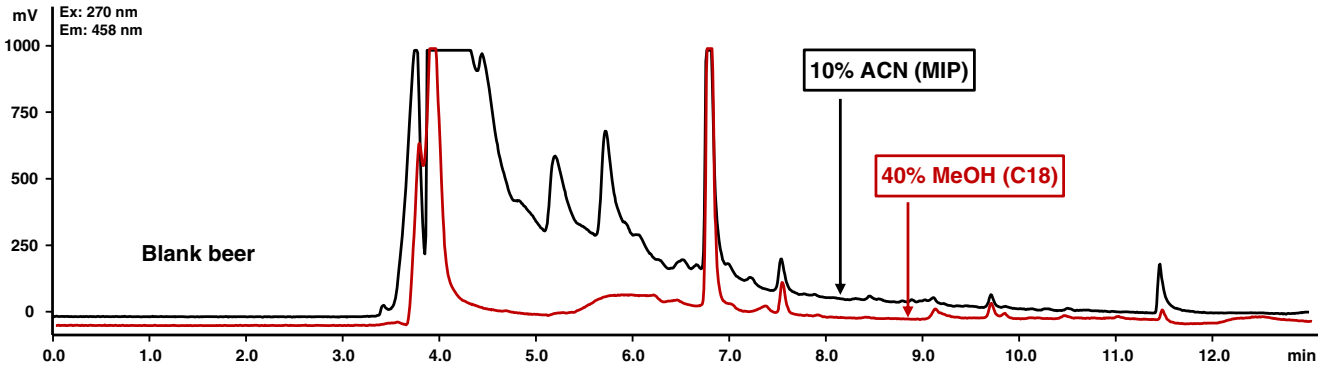

Fig. 4 Comparison of beer matrix clean-up efficiency on MIP sorbent (black line) and C18 sorbent (red line) with upper limit of organic solvents in washing mobile phase

chromatogram of the blank beer and ZEA spiked beer samples under the optimal conditions of extraction and separation.

Validation of on-line SPE-HPLC methods

Both on-line extraction methods using the MIP and the C18 sorbent were validated in terms of linearity, selectivity, precision, accuracy, and detection limits. The validation characteristics were compared for evaluating method equivalency. A test of the system suitability parameters was included, and peak capacity, symmetry, and repeatability were calculated from six injections of standard mixture at three different concentration levels 200, 100, and 20 μg L^{-1}. All parameters are summarized in Table 1. The retention times and retention factors were the same due to the same linear gradient program for elution. Extraction using the C18 sorbent provided better peak symmetry and repeatability (both methods RSD < 1.3%).

Eight-point calibration curve was plotted using results achieved with standard solutions having concentrations of 5–200 μg L^{-1} dissolved in water and in blank beer for matrix calibration. The lowest concentration of the calibration curve, 5 μg L^{-1}, was set as the limit of quantification (LOQ). The limit of detection (LOD) was calculated from the relationship between LOQ and LOD, which are equal to a signal-to-noise ratio of ten and three times, respectively. Additionally, the calculated LOD concentration of 1.5 μg L^{-1} was confirmed experimentally by injecting additional diluted spiked beer.

The precision of both methods, including on-line sample pretreatment, was determined as relative standard deviation (RSD, %) using repetitive injections of seven spiked beer samples at a concentration of 50 μg L^{-1}. The resulting RSD values were 2.5 and 5.4% for MIP and C18, respectively. The accuracy expressed as percentage recovery was calculated as the ratio of the mean peak area of seven blank beer samples spiked at a concentration of 50 μg L^{-1} and the peak area of the standard solution at the same concentration ($\% = \frac{\text{AUC spike}}{\text{AUC stand}} \cdot 100$). Calculated recoveries close to 100% indicated no matrix interferences. Complete results of validation are shown in Table 2. Both methods were compared in term of selectivity of on-line extraction step. The critical evaluation did not indicate any increased selectivity of on-line solid-phase extraction based on a molecularly imprinted polymer (MISPE). In fact, since zearalenone is rather lipophilic, it was strongly retained on the C18 extraction sorbent via hydrophobic interactions, which enabled the use of a washing mobile phase with a strong elution power, thus leading to a more efficient clean-up of the mass of the matrix ballast. The comparison of clean-up efficiency of both techniques is presented in Fig. 4.

Selective recognition of the analyte of choice by MIP sorbent depends, among other things, on the nature of the binding

Table 1 System suitability parameters of two on-line SPE-HPLC methods for zearalenone determination using C18 and MIP extraction sorbents

	Retention time (min)[a]	Peak capacity[b]	Peak symmetry[c]	Repeatability of peak area, (RSD %)[d]
MISPE-HPLC	7.78	33.84	1.61	1.3; 1.2; 1.2
(C18)-SPE-HPLC	7.78	33.84	1.39	0.7; 0.5; 0.3

[a] Retention time of zearalenone

[b] Peak capacity expressing efficiency of method (gradient elution) is calculated as P_c = (the gradient time/4 × peak width in half) + 1 (times did not include on-line SPE step)

[c] Peak symmetry was calculated by the Lab Solution software (ratio of descending to ascending part of peak in 10% of high)

[d] RSD was calculated from six injections of standard mixture at concentration levels: c_1 = 200 μg L^{-1}, c_2 = 100 μg L^{-1}, c_3 = 20 μg L^{-1}

Table 2 Analytical characteristics of the validated on-line SPE-HPLC methods (comparison of C18 and MIP sorbent)

	C18	MIP
Standard linear calibration range (μg L^{-1})[a]	5–200	5–200
Slope	9997 ± 430	16,705 ± 206
Intercept	99,325 ± 43,358	−1429 ± 20,751
Regression coefficient (r^2)	0.9945	0.9995
Matrix (beer) linear calibration range (μg L^{-1})[a]	5–200	5–200
Slope	13,853 ± 463	17,897 ± 403
Intercept	9193 ± 46,583	32,365 ± 40,558
Regression coefficient (r^2)	0.9967	0.9985
LOD (μg L^{-1})	1.5	1.5
LOQ (μg L^{-1})	5	5
Precision (RSD, %) in beer[b]	5.4	2.5
Accuracy-spike recovery (%) ± SD in beer	100.1 ± 5.4[c]	99.0 ± 2.5[c]

[a] Each concentration level was measured in triplicate

[b] Repetitive determination of seven spiked beer samples at one concentration level 50 μg L^{-1}

[c] Accuracy was determined as a method recovery using seven spiked beer sample at concentration level 50 μg L^{-1}, each in triplicate (± minimal and maximal standard deviation of recovery determination)

interactions. Rebinding kinetics and fast desorption are also important. For that reason, non-covalent imprinting is the most frequently used technique for MIP preparation. Template binding by non-covalent interactions including π-π, hydrogen-bonding, and electrostatic interactions is most versatile and affords materials with faster kinetics. The accessibility of binding sites plays an important role, in addition to the swelling of the polymer in a solvent and its porosity that are also important features. Because rebinding depends on the medium, the washing mobile phase was optimized with respect to acetonitrile and acetic acid as it was recommended for zearalenone extraction. However, contrary to the manufacturer instruction sheet, Lucci et al. [22] proposed that for specific interactions, sorbent drying and switching to a non-aqueous washing step might be necessary. In our case, we could only achieve non-specific interactions with the on-line approach. Hydrophobic interactions prevailed in the aqueous mobile phases and MIP behaved more like a reversed-phase sorbent or a non-imprinted polymer. This effect correlates well with our experimental results.

In the case of decreased specific interactions, an MIP sorbent is not a very convenient solution to the adsorption and extraction due to the lower than required selectivity. The commercial polymer is available as particles with a broad 25–80 μm particle size distribution (certificate of quality control by Affinisep). In contrast, C18 sorbent used for comparison contained 5 μm core-shell particles. In accordance with the peak symmetry of zearalenone and system suitability test results (see Table 1, Fig. 3), solid core of the particles, narrow distribution of their size, and short diffusion path length within the sorbent improved the efficiency of the reversed-phase extraction process. As a result, the reversed-phase-based SPE-HPLC analysis should be the method of choice particularly when handling nonpolar lipophilic analytes in an aqueous matrix such as beer. For clarity, the chromatograms of blank beer and ZEA spiked beer samples under the optimal and validated conditions for both extraction approaches are compared in Fig. 3.

Beer sample analyses

Thirty beer samples were analyzed (details are in Table S1 in the Electronic Supplementary Material (ESM)). Furthermore, 50 μL of untreated beer was injected in system and on-line extraction and separation were performed in a single run. Zearalenone was detected in only three beers, in both cases in concentration under the limit of quantification and far under the strictest permitted concentration of 20 μg L^{-1} (European Commission Regulation No 1126/2007) [14]. Therefore, the Czech beers do not represent a serious risk of ZEA contamination for consumers.

Conclusions

Two novel chromatographic methods for zearalenone determination in beer including an on-line extraction step have been developed, validated, and compared. Molecularly imprinted solid-phase extraction on-line coupled to column-switching HPLC system for zearalenone determination was presented for the first time. The possibility to achieve a specific interaction between an analyte and MIP using the on-line connection directly in a chromatography system was studied. Alternatively, a non-specific C18 extraction sorbent was used for comparison. Chromatograms, sample clean-up efficiency, selectivity, and validation parameters were evaluated.

According to the obtained validation results, both methods were comparable and no significant difference in selectivity was observed. The reason could be that only non-specific hydrophobic interactions are achieved while using the on-line technique. It was not possible to apply all steps and solvents primarily optimized for off-line work, such as drying out the sorbent and switching to non-aqueous conditions. These features are probably necessary to achieve the specific interactions. In our case, ZEA, which is rather lipophilic, was more strongly retained on the C18 sorbent. Therefore, a better clean-up of polar matrix interferences was achieved during the on-line extraction step. In addition, the C18 reversed-phase sorbent consisted of core-shell particles with narrow particle size distribution and the ZEA peak was more symmetrical. In conclusion, the high selectivity of the MIP extraction process coupled on-line to chromatography system was not confirmed. Using our method, 30 Czech beers were analyzed for ZEA contamination and only 3 samples turned positive but the contents were negligible.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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