Journal of Chromatography A, 1217 (2010) 6668–6673

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Solid-phase extraction using molecularly imprinted polymers for selective extraction of a mycotoxin in cereals

Wassim Hadj Ali[a,b], Delphine Derrien[b], Florent Alix[b], Céline Pérollier[b], Olivier Lépine[b], Sami Bayoudh[b,*], Florence Chapuis-Hugon[a], Valérie Pichon[a]

[a] *Department of Environmental and Analytical Chemistry (UMR PECSA 7195 CNRS-UPMC Paris 06-ESPCI ParisTech), ESPCI ParisTech, 10 rue Vauquelin, 75231 Paris cedex 05, France*
[b] *POLYINTELL, Pharmaparc II, Voie de l'innovation, 27100 Val de Reuil, France*

ARTICLE INFO

Article history:
Available online 18 May 2010

Keywords:
Molecularly imprinted polymer
Solid-phase extraction
Mycotoxin
Food analysis

ABSTRACT

The aim of this work was to develop a method for the clean-up of a mycotoxin, *i.e.* Ochratoxin A (OTA), from cereal extracts employing a new molecularly imprinted polymer (MIP) as selective sorbent for solid-phase extraction (SPE) and to compare with an immunoaffinity column. A first series of experiments was carried out in pure solvents to estimate the potential of the imprinted sorbent in terms of selectivity studying the retention of OTA on the MIP and on a non-imprinted polymer using conventional crushed monolith. The selectivity of the MIP was also checked by its application to wheat extracts. Then, after this feasibility study, two different formats of MIP: crushed monolith and micro-beads were evaluated and compared. Therefore an optimization procedure was applied to the selective extraction from wheat using the MIP beads. The whole procedure was validated by applying it to wheat extract spiked by OTA at different concentration levels and then to a certified contaminated wheat sample. Recoveries close to 100% were obtained. The high selectivity brought by the MIP was compared to the selectivity by an immunoaffinity cartridge for the clean-up of the same wheat sample. The study of capacity of both showed a significant higher capacity of the MIP.

1. Introduction

Mycotoxins have received an increasing interest from scientific community in food safety. Indeed, their impact on human health is still considered as hazardous. Ochratoxin A (OTA) is the most abundant naturally occurring mycotoxin produced by mainly *Penicillium verrucosum* and *Aspergillus ochraeus* that can contaminate food commodities prior to harvest or more commonly during storage [1–3]. Therefore, OTA can be found as a natural contaminant in cereals [1,4,5], wine [6,7], grape juice, coffee beans [8–10], cacao [11], spices and dried wine fruit [12]. In the European diet, cereals such as barley, wheat, maize, oats and their by-products are considered as the major source of OTA intake (corresponding to 50%) [12]. The monitoring of OTA in food must help in the limitation of its consumption by ingestion. OTA inhibits protein synthesis and lipid peroxidation by oxidative processes and these mechanisms may generate nephrotoxic, neurotoxic and immunotoxic effects [12–14]. OTA is then considered by the International Agency for Research on Cancer as possibly carcinogenic for humans (group 2B), and teratogenic and carcinogenic effects have been also described in some animal species [2,15]. However it is well-known that OTA is chemically stable so it tends to survive to storage and processing, and is not destroyed when cooked at quite high temperatures such as those reached during baking bread of breakfast cereal production [16,17].

To protect human and animal exposure to OTA, the European Union (EU) enacted a regulatory limit for its levels depending on the food: cereals (5 $\mu g\,kg^{-1}$), roasted coffee (5 $\mu g\,kg^{-1}$) or instant coffee (10 $\mu g\,kg^{-1}$), all products derived from cereals (3 $\mu g\,kg^{-1}$) and processed cereal-based foods and baby-foods (0.5 $\mu g\,kg^{-1}$). Therefore, there is an important request for fast, reliable and low-cost analytical methods for the monitoring of OTA in food.

The conventional methods used for the identification of OTA are based on the use of liquid chromatography and fluorescence detection, the native fluorescence of OTA favoring the development of a very sensitive method [3]. This conventional analytical method is currently associated with the use of an immunoaffinity column (IAC) for the sample treatment to remove matrix components. IAC are composed of anti-OTA antibodies (Abs) covalently immobilized on a solid-sorbent. The use of IAC was validated and largely applied to improve the reliability of the analysis of foodstuff [1,4,5,8–11] but also plasma [2] or urine [18] samples. These numerous appli-

* Corresponding author. Tel.: +33 2 3209 3270; fax: +33 2 3259 6101.
E-mail address: sami.bayoudh@polyintell.com (S. Bayoudh).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.04.071

cations highlight the great interest of using immunoaffinity for sample pretreatment.

IACs are already commercialized (Ochraprep™ from R-Biopharm Rhône, OchraTest™ from Vicam) and their use was validated by many organisations such as the Association of Official Analytical Chemists (AOAC) and EU. Two methods are also in process for their normalization by AFSSA (PR NF EN 15829, PR NF EN 14133) for wines, grapes, dried wine fruit and beer. At last, IAC methods notably in the case of OTA could induce underestimation of the real concentration of OTA in the samples [16,17].

The aim of this work is to propose more powerful and less expensive extraction devices dedicated to OTA and to its metabolites and adducts, as an alternative to IAC.

It consists in the use of molecularly imprinted polymer (MIP) having specific cavities that are therefore considered as antibody mimics, the cavities allowing a selective trapping of the target analytes as the recognition sites of the Abs. Taking into account the price of available cartridges of IAC and of MIP we can presume that those materials are less cost-effective to develop than Abs.

Molecularly imprinted polymers (MIPs) may present an interesting alternative to these established materials, as they often exhibit very selective analyte retention, and do not suffer from storage limitations and stability problems regarding organic solvents [19–22]. They are also characterized by a higher capacity than IAC, *i.e.* a higher amount of a target analyte being retained on MIP than on IAC when comparing similar amount of sorbent packed in a SPE cartridge [23]. Because of these reasons, MIPs bear considerable promise to complete the range of SPE materials for analyte-selective sample preparation. These sorbents are synthetic polymeric materials possessing specific cavities designed for a template molecule involving a retention mechanism based on molecular recognition.

MIPs specifically designed for OTA have been already developed using directly OTA as template [24] or using a structural analog [25–27] to circumvent the problem of template leaking during SPE. Several analogs were studied and L-Phe-CHNA (N-(4-chloro-1-hydroxy-2-naphtholylamido)-(L)-phenylalanine) seems to generate cavities having a strong affinity for OTA. Concerning the choice of monomers, computational design methods modeling the interactions of OTA with a virtual library of monomers (computational design method) [24] or results of experiments consisting in preparing various polymers with different types of monomers [26] highlight the necessity to use monomers having both basic and hydrophobic properties.

Most of the studies related to MIP for OTA reported the high selectivity of this sorbent in pure media [24–26]. Nevertheless the potential of MIPs has to be confirmed for the treatment of wines, cereal extracts, etc. Indeed, a MIP for OTA prepared by electrochemical polymerization of pyrrole onto a stainless steel frit and using carbon nanotubes as nanostructured fibers was used for the on-line preconcentration of the mycotoxin from wines [28,29]. A very low limit of quantification was announced (41 ppt) but unfortunately no real selectivity brought by the MIP was clearly demonstrated. Another study has reported the direct extraction of OTA from wine using MIP and matrix effects were revealed by a decrease of recoveries comparing to recoveries obtained in pure media [27]. These problems of matrix effects have been largely reported in the literature and it is now well-known that a careful optimization of the conditions of use may circumvent matrix effects [30]. So it is necessary to control the nature of the interactions involved in the retention process that could help to develop extraction procedure that does not suffer from matrix effects [21–23,31,32].

The aim of this work was to use a MIP targeted to OTA for its application in the treatment of complex matrices such as cereal extracts. A first solid-phase extraction characterization of a conventional crushed monolith MIP was achieved in a non-protic solvent, in order to demonstrate the presence of selective cavities in the MIP. The selectivity of the developed procedure was assessed by studying the retention of the target analyte on the NIP. Then, the use of MIP beads was considered as a very valuable strategy; the bulk polymerization produces monoliths which need to be ground before being sieved and used, giving highly irregular and heterogeneous morphologies on contrary to homogeneous and spherical beads. These steps are man-power consuming and generate a significant loss of materials whereas beads synthesis only requires extraction of the target molecule before being used with a very high ratio of usable materials. So, the bead process is fully adapted for the commercialization of the final product by increasing its competitiveness with a more efficient and cheaper product.

The evaluation of these beads was achieved by studying the retention of OTA during its extraction from wheat extracts. The optimized extraction procedure was also applied to spiked wheat samples and certified contaminated wheat samples and results were compared to those obtained using an immunoaffinity sorbent.

2. Experimental

2.1. Chemicals

OTA standard was supplied by Sigma (Steinheim, Germany). A standard solution of 50 mg L^{-1} was prepared by weighting the solute and dissolving it in methanol. Another standard solution of 5 mg L^{-1} was obtained by dilution in methanol from the standard solution. These solutions were stored at 4 °C. Certified reference material was provided by LGC standards (Molsheim, France). HPLC-grade acetonitrile and methanol were purchased from Carlo Erba (Val de Reuil, France). Acetic acid (AA) was purchased from Sigma (Steinheim, Germany), hydrochloric acid (HCl) from Carlo Erba (Val de Reuil, France), ammonia solution (NH_3) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Sodium chloride (NaCl) was purchased from Sigma (Steinheim, Germany). Potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Alfa Aesar (Karlsruhe, Germany). 8 g of NaCl, 1.2 g of Na_2HPO_4, 0.2 g of KH_2PO_4 and 0.2 g of KCl were mixed in 1 L of water to prepare a phosphate buffer saline (PBS).

Molecularly imprinted polymer crushed monolith and bead (AFFINIMIP™ OTA) and non-imprinted polymer (NIP) were provided by POLYINTELL (Val de Reuil, France). MIPs are obtained by radical polymerization using initiator 2,2′-azobis-isobutyronitrile (Aldrich) and based on difunctional acrylic cross-linker monomers (Aldrich). Immunoaffinity cartridges (Puri-Fast OTA IAC) were purchased from LIBIOS (Bully, France).

2.2. Apparatus and analytical conditions

Two HPLC systems were used. The first one used for the evaluation of crushed monolith MIP consisted in a Waters 717 autosampler (Waters, Saint-Quentin en Yvelynes, France) and a Varian Prostar system, including a solvent delivery system (Prostar 230) and a fluorescence detector (Prostar 330) (Varian, Les Ulis, France). OTA was monitored at λ_{ex} 334 nm and λ_{em} 460 nm. The reversed-phase column was a Waters SunFire™ C18 5 μm 150 mm × 4.6 mm i.d. (Agilent Technologies, Massy, France) that was connected to a precolumn filter (2 μm frit, Upchurch Scientific, Oak Harbour, WA, USA). The mobile phase consisted in a mixture of acetonitrile/water (60:40, v/v) containing 1% of acetic acid. The flow rate was set at 0.2 mL min^{-1} and the sample injection volume was 20 μL.

The second one used for the evaluation of MIP beads consisted in a ThermoFinnigan Spectra System, including a solvent delivery

Table 1
Repeatability of the recoveries of extraction on MIP beads obtained on wheat extracts spiked with OTA, $n = 3$.

Spiking level ($\mu g\,kg^{-1}$)	Recoveries (%)	RSDr (%)
2.5	102.7	1.5
5	82.5	2.5
100	86.9	4.5

RSDr: repeatability relative standard deviation.

system (P1000XR), an autosampler (AS3000) and a Jasco fluorescence detector (FP-2020Plus) (Jasco, Bouguenais, France). OTA was monitored at λ_{ex} 333 nm and λ_{em} 460 nm. The reversed-phase column was a Hypersil Gold C18 3 μm 150 mm × 2.1 mm i.d. protected by a precolumn Hypersil Gold C18 3 μm 10 mm × 2.1 mm i.d. both from Thermo (Bellefonte, Pennsylvania, USA). The mobile phase consisted in a mixture of water–MeOH (40/60, v/v) containing 1% of acetic acid. The flow rate was set at 0.2 mL min^{-1} and the sample injection volume was 20 μL.

2.3. Study of the retention mechanism in pure media

Cartridges of 1 and 3 mL were packed with 50 and 100 mg of MIP or NIP respectively. Before each use, the sorbent was conditioned with a few millilitres of acetonitrile. For the 50 mg and the 100 mg cartridges, 1 or 2 mL of acetonitrile respectively were spiked with OTA (500 ng) and were percolated through the MIP/NIP cartridges. Then, the sorbent was washed with an acetonitrile/methanol (30/70) mixture (4 or 8 mL for 50 or 100 mg of polymer respectively) and with methanol (1 or 2 mL for 50 or 100 mg of polymer respectively). The target analyte was finally eluted from the cartridge with an acetonitrile/methanol/acetic acid (45/45/10) mixture (1 or 2 mL for 50 or 100 mg of polymer respectively). Each fraction was then diluted by a factor 2 with water. 20 μL were analyzed by reversed-phase liquid chromatography (RP-LC).

2.4. Extraction of OTA from real matrices

50 g of wheat was extracted using a blender with 100 mL of ACN/water (60/40, v/v). The resulting extract was filtered. For the validation study (Table 1), MIP cartridges (100 mg) containing 25–80 μm beads were conditioned with 5 mL ACN and 5 mL water. The wheat extract was diluted by a factor 2 with an aqueous HCl solution 0.1 M, spiked with different amounts of OTA and 4 mL were percolated through the MIP. The washing procedure consisted of 1 mL of HCl 0.1 M, 1 mL of acidified water (HCl 0.1 M)/ACN 60/40 (v/v), 10 mL of purified water, a drying step of 5 min, 4 mL of ACN containing 0.01% AA. The elution was carried out with 2 mL of MeOH containing 2% AA. The elution fraction was diluted by a factor 2 with water and 20 μL were injected in LC/Fluo. The same procedure was applied for the treatment of the certified sample.

When using immunoaffinity (IA) cartridges, 10 mL of wheat extract were diluted with 40 mL of water, the resulting solution was filtered and 10 mL were percolated on the IA cartridge. The sorbent was washed with 10 mL of PBS (pH = 7) and 10 mL of pure water. The elution was carried out with 1.5 mL of MeOH. The resulting fraction was diluted with water (factor 2) and 20 μL were analyzed in LC/Fluo.

3. Results and discussion

3.1. Study of retention of OTA on the crushed monolith MIP

Firstly, the MIP was characterized in a non-protic and weakly polar solvent thus favoring the expected selective interactions between the target molecule and the MIP as described in Section 2.3. Therefore, ACN samples spiked with OTA were used to demonstrate an imprinting effect of the polymer (Fig. 1).

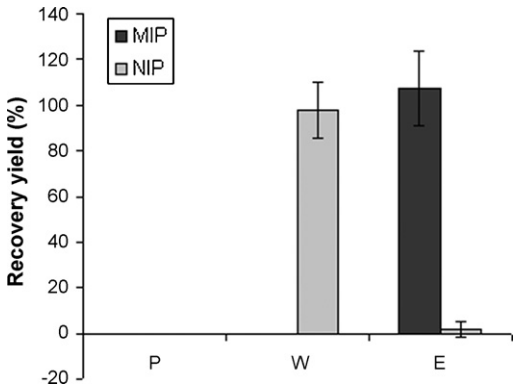

Fig. 1. Elution profile ($n = 3$) on MIP and on NIP (cartridges of 50 mg) after the percolation 1 mL of ACN spiked with 500 ng of OTA (P). Washing (W): 4× 2 mL of ACN/MeOH 30/70 (v/v) + 1 mL MeOH; elution (E) with 1 mL ACN/MeOH/AA 45/45/10 (v/v/v). LC/Fluo analysis of each fraction.

Thus, 1 mL of MeCN spiked with 500 ng of OTA was percolated through 50 mg of MIP packed in a disposable cartridge. The same experiment was carried out on the NIP in order to control and evaluate the risk of the development of the non-specific interactions in the retention process. To remove OTA from the NIP during the washing step and to obtain a high degree of selectivity, the content of washing solutions was optimized. The role of this step was to disrupt the residual non-specific interactions formed on the NIP by hydrogen bonds. It ensures that the retention process results from the presence of cavities. The optimized washing procedure consists of the percolation of 4 mL of an ACN/MeOH mixture (30/70, v/v) and then 1 mL of MeOH. The elution was ensured by the percolation of 1 mL of a mixture of ACN/MeOH/AA (45/45/10, v/v/v). A high extraction recovery of 107 ± 16% was obtained on the MIP with a low recovery of 2% on the NIP. This very satisfactory result clearly demonstrates the real imprinting effect of the MIP.

When optimizing the elution conditions, the addition of AA in the eluting fraction appears absolutely necessary for a complete elution, thus highlighting the contribution of electrostatic interactions in the retention procedure.

The final objective of this work was to develop a selective sorbent that can be developed at a large scale and that can be applied in routine to complex matrices such as cereals extracts. Then, this sorbent was tested on a cereal extract in order to check that the matrix content does not affect the selective retention of OTA. Indeed, it was largely mentioned that matrix components may modify the nature of the interactions developed between the target analyte and the MIP thus causing retention properties.

Therefore, an extract corresponding to 1 g of wheat diluted in an ACN/water (55/45, v/v) mixture and spiked with 100 ng of OTA was percolated through the MIP and the NIP. The dilution of the wheat extract was achieved in a hydro-organic mixture because it is close to the nature of the solvent generally used for OTA extraction from cereals.

The elution profiles obtained on MIP and on NIP when applying the previously described procedure of washing and elution are presented in Fig. 2. The volume of the different solution of washing and of elution was adapted to the amount of sorbent (100 mg instead of 50 mg packed in each cartridge). These results show that the retention of OTA is slightly affected by matrix components particularly on NIP because a large amount of OTA was found in the percolation fraction. In return, the retention on the MIP is still high with 93% of recovery in the elution fraction.

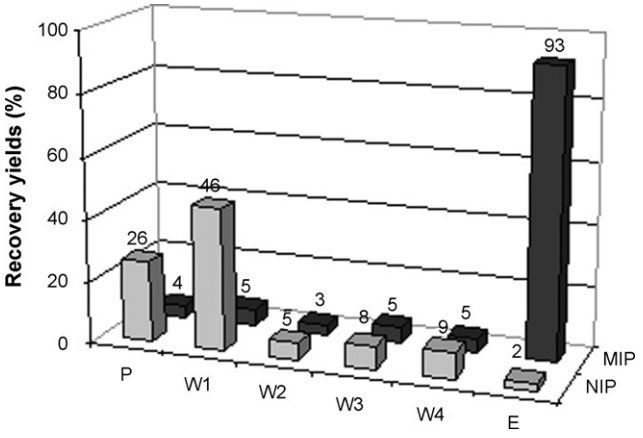

Fig. 2. Elution profiles obtained on MIP and on NIP (100 mg of sorbent) after the extraction of 1 g of wheat (extraction with ACN). P: percolation of the extract diluted in ACN (1.6 mL), spiked with 100 ng of OTA diluted with 1.3 mL of water, W1: 2 mL ACN, W2 and W3: 3 mL ACN/MeOH (50/50, v/v), W4: 3 mL MeOH and E: 2 mL ACN/MeOH/AA (45/45/10, v/v/v).

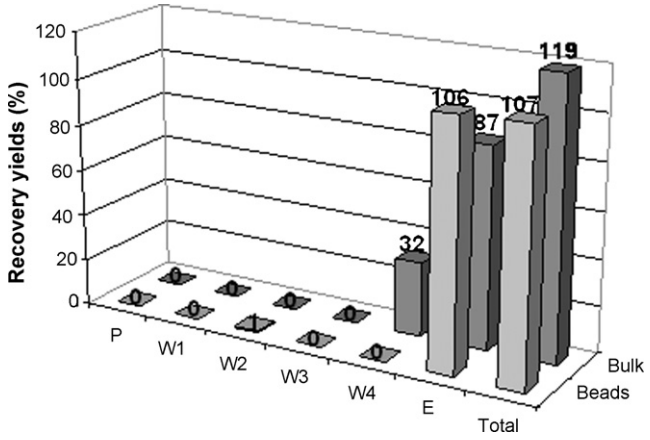

Fig. 4. Elution profile obtained using MIP crushed monolith or MIP beads. Percolation: 1 mL of wheat extract (60/40 ACN/water) diluted by 4 with water (equivalent to 0.125 g of wheat) containing 100 ng of OTA; washing steps: 1 mL water (W1), 1 mL ACN (W2), 1 mL ACN/MeOH (85/15, v/v) (W3, W4), elution with 2 mL ACN/MeOH/AA (45/45/10, v/v/v). A drying step of 15 min was introduced between W1 and W2. Fractions were analyzed by LC/Fluo.

The chromatograms corresponding to the analysis of the washing and the elution fractions are compared in Fig. 3A. The presence of a huge peak in the beginning of the chromatograms corresponding to the analysis of the washing fraction illustrates the efficiency of the washing steps. This allows the removal of interfering compounds from the MIP thus providing a clean chromatogram when analyzing the elution fraction. The analysis of the elution fraction resulting from the NIP confirms the absence of retention of OTA on this sorbent and then the selectivity of the extraction procedure of the MIP owing to the presence of specific cavities. All these results have shown the feasibility to develop a MIP for the selective extraction of OTA. This work goes then on the evaluation of beads to obtained well-defined and controlled particles of MIP.

3.2. Evaluation of MIP beads

3.2.1. Comparison between MIP crushed monolith and MIP beads

In order to compare MIP crushed monolith and MIP beads, both types of MIP particles (100 mg) were packed in a cartridge and the retention of OTA on both sorbent was evaluated by percolation a wheat extract spiked with OTA. Resulting elution profiles and conditions are described in Fig. 4.

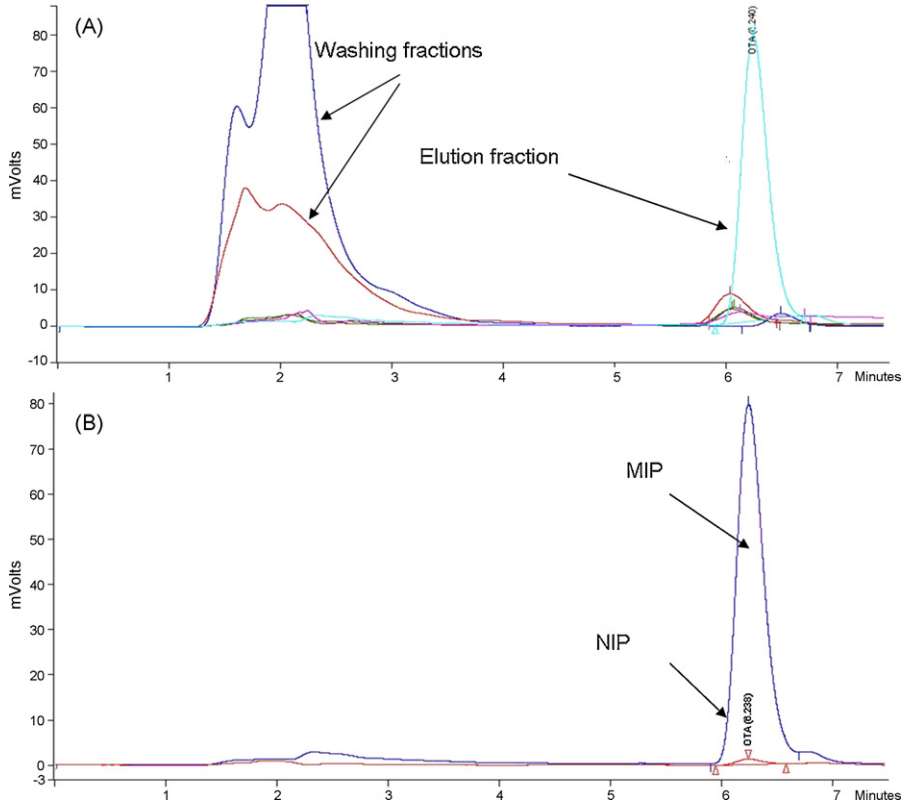

Fig. 3. LC/Fluo analysis of the percolation, washing and elution fraction from the MIP after the treatment of the wheat extract (A) and comparison of the elution fraction obtained with the MIP and the NIP (B). Conditions: see Fig. 2.

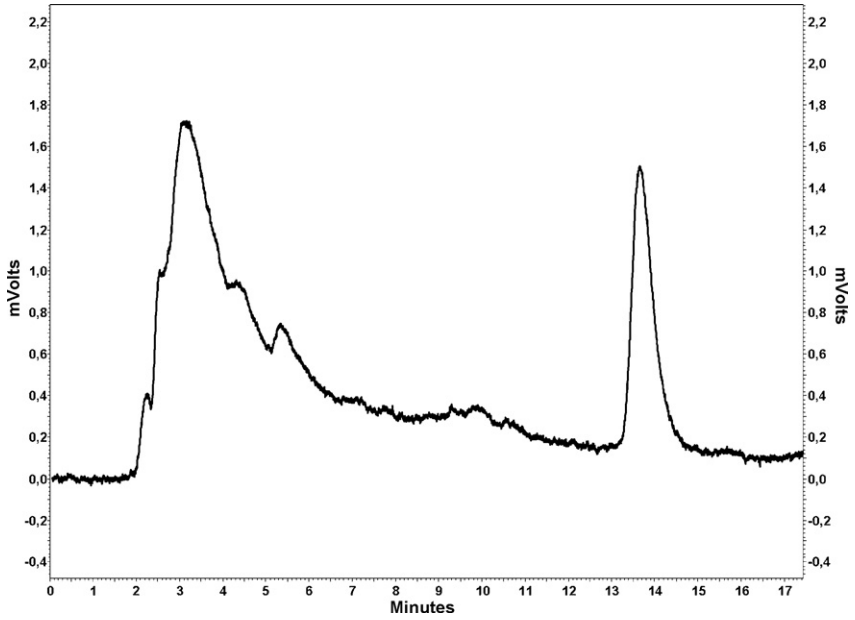

Fig. 5. LC/Fluo analysis of the certified matrix reference material (B-myc0880) after a purification step on MIP beads (see conditions in Section 2.4).

This protocol showed a difference between MIP crushed monolith and MIP beads. The loss of OTA in the last washing fraction corresponding to the crushed monolith MIP and the absence of OTA in the washing fraction resulting from the use of beads indicate the strongest retention of OTA on the MIP beads. Nevertheless, impurities were still present in the elution fractions. These results explained why it was necessary to optimize the protocol to limit the presence of impurities in the elution fraction resulting from the use of this more retentive sorbent.

3.2.2. Validation of the extraction procedure on MIP beads

The validation of the whole analytical method was carried out by the purification on MIP beads of wheat extract samples spiked at 2.5, 5 and 100 $\mu g\,kg^{-1}$ and by applying the extraction procedure previously described in Section 2.4. Recoveries obtained for each sample are reported in Table 1. Values are in the range 86.9–102.7% with RSD values between 1.5 and 4.5%. Those good results highlight the performance of the method for the quantitative analysis of wheat samples.

This validation study was rounded off by the application of the extraction procedure to a certified matrix reference material (B-myc0880). The certified value was $2.7 \pm 1.0\,\mu g\,kg^{-1}$. The concentration value found for the analysis of this material ($n = 2$) was 2.8 and 2.9 $\mu g\,kg^{-1}$ thus corresponding to an extraction recoveries of 102 and 108% respectively. The chromatogram corresponding to the injection of the elution fraction after the purification on MIP bead is presented in Fig. 5. A correct baseline was obtained thus

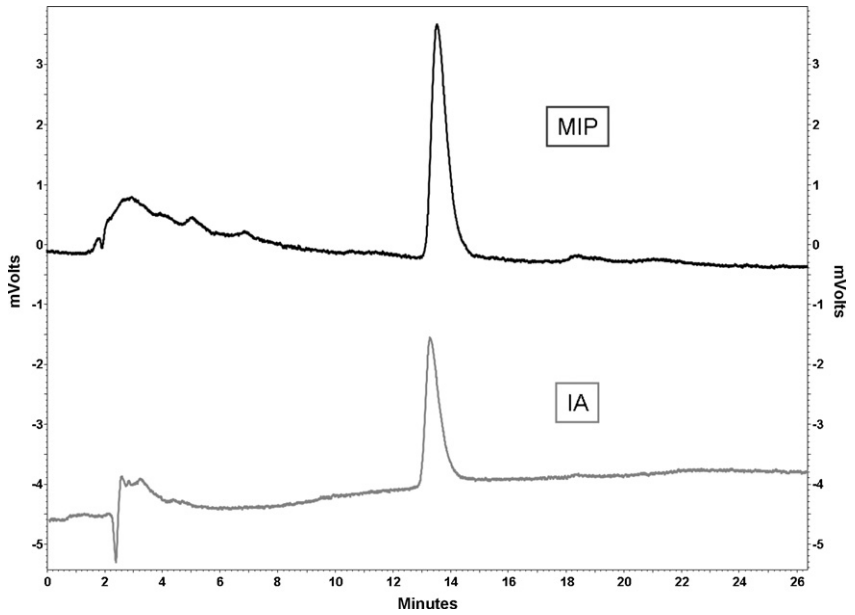

Fig. 6. Comparison of the elution fractions obtained with the MIP and the IA cartridges after the extraction of 1 g of wheat, spiked respectively with 10 ng of OTA per g of wheat for the MIP and with 6 ng of OTA per g of wheat for the IAC.

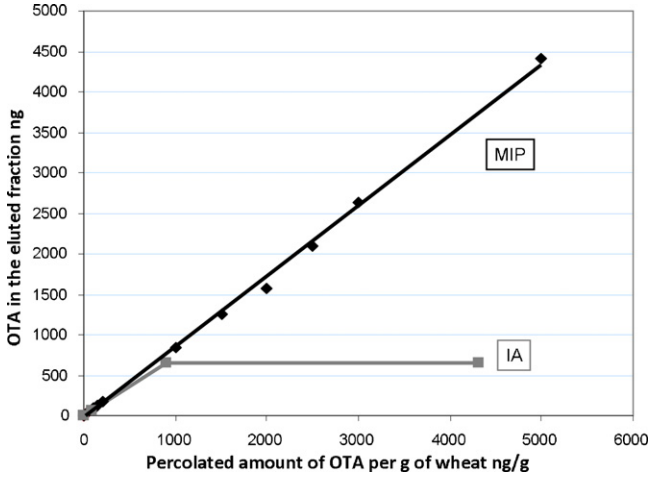

Fig. 7. Amount of OTA (ng) quantified in the elution fraction from the MIP and from the IA as a function of the amount of OTA in the percolated extract per g of wheat.

allowing an easy quantification of OTA in this contaminated wheat samples.

3.3. Comparison of MIP beads to immunoaffinity cartridge

As previously mentioned, the conventional analytical method applied for the determination of OTA in foodstuff consists of the purification of the extract on an immunoaffinity cartridge (IAC) to remove matrix components before LC/Fluo analysis. IAC are composed of anti-OTA antibodies (Abs) covalently immobilized on a solid-sorbent. Therefore, the potential of MIP was also evaluated by comparing chromatograms obtained by injecting the extracts resulting from MIP and IA purification of spiked wheat samples. These chromatograms are presented in Fig. 6. Very reliable baselines were obtained in both case thus highlighting the high potential in selectivity of the biological and the biomimetic sorbents. Similar recoveries were obtained with 85% on MIP and 79% on IA. To complete the comparison of these sorbents, the capacity of both sorbents were compared. The capacity of a selective sorbent corresponds to the maximum amount of a compound that can be retained on this sorbent in given conditions. It depends for MIP of the number of specific cavities and of specific recognition sites (number of immobilized antibodies) for immunosorbent. Therefore, the determination of the capacity of both sorbents was performed by measuring the extraction recoveries of OTA after the percolation of increasing amount of OTA and the application of the extraction procedure was adapted to each sorbent.

Fig. 7 shows the results obtained when plotting the amount of OTA measured in the elution fraction of the MIP and of the IA as a function of the percolated amount.

For the MIP, the recoveries of the extraction were linear up to 5000 ng g^{-1}, whereas for IAC when percolating an extract containing 900 ng or 4300 ng g^{-1} of OTA, only 650 ng are detected in the elution fraction of the IA. These results indicate a decrease of recoveries on IA caused by the overloading of the capacity.

4. Conclusion

This work propose the suitability of the developed water-compatible MIP-SPE procedure for the analysis of Ochratoxin A (OTA), from cereal extracts employing a new molecularly imprinted polymer (MIP) as selective sorbent for solid-phase extraction (SPE). In addition, the MIP-SPE technique has the advantage to be selective, cost-effective, chemically and thermally stable. The developed and validated method shows satisfactory linearity, precision and accuracy. The comparison with IAC shows similar selectivity results and the capacity study demonstrates the high potential of this new selective sorbent MIP.

References

[1] B. Kabak, Food Chem. Toxicol. 47 (2009) 348.
[2] K. Muñoz, M. Vega, G. Rios, S. Muñoz, R. Madariaga, Food Chem. Toxicol. 44 (2006) 1884.
[3] N.W. Turner, S. Subrahmanyam, S.A. Piletsky, Anal. Chim. Acta 632 (2009) 168.
[4] C. Zaied, S. Abid, L. Zorgui, C. Bouaziz, S. Chouchane, M. Jomaa, H. Bacha, Food Control 20 (2009) 218.
[5] P. Villa, P. Markaki, Food Control 20 (2009) 455.
[6] D. Flajs, A.M. Domijan, D. Ivic, B. Cvjetkovic, M. Peraica, Food Control 20 (2009) 590.
[7] G.J. Soleas, J. Yan, D.M. Goldberg, J. Agric. Food Chem. 49 (2001) 2733.
[8] A. Napolitano, V. Fogliano, A. Tafuri, A. Ritieni, J. Agric. Food Chem. 55 (2007) 10499.
[9] N.E. Ahmed, M.M. Farag, K.M. Soliman, A.K.M. Abdel-Samed, K.M. Naguib, J. Agric. Food Chem. 55 (2007) 9576.
[10] A. Pittet, D. Tornare, A. Huggett, R. Viani, J. Agric. Food Chem. 44 (1996) 3564.
[11] J. Serra Bonvehi, J. Agric. Food Chem. 52 (2004) 6347.
[12] A. Pfohl-Leszkowicz, R.A. Manderville, Mol. Nutr. Food Res. 51 (2007) 61.
[13] A. Pfohl-Leszkowicz, M. Tozlovanu, V. Faucet-Marquis, M. Peraica, V. Stefanovic, R. Manderville, Mutagenesis 23 (2008) e1.
[14] P.G. Mantle, V. Faucet-Marquis, R.A. Manderville, B. Squillaci, A. Pfohl-Leszkowicz, Chem. Res. Toxicol. 23 (2010) 89.
[15] P. Mosesso, S. Cinelli, J. Pinero, R. Bellacima, G. Pepe, Chem. Res. Toxicol. 21 (2008) 1235.
[16] A. Pfohl-Leszkowicz, A. Molinié, M. Castegnaro, Rev. Mex. Micol. 19 (2004) 7.
[17] M. Castegnaro, M. Tozlovanu, C. Wild, A. Molinié, A. Sylla, A. Pfohl-Leszkowicz, Mol. Nutr. Food Res. 50 (2006) 480.
[18] M. Pascale, A. Visconti, Mycopathologia 152 (2001) 91.
[19] L. Amalric, C. Mouvet, V. Pichon, S. Bristeau, J. Chromatogr. A 1206 (2008) 95.
[20] F.B. Kaabi, V. Pichon, LC–GC North Am. 25 (2007) 732.
[21] F. Chapuis, J.U. Mullot, V. Pichon, G. Tuffal, M.C. Hennion, J. Chromatogr. A 1135 (2006) 127.
[22] M. Lasáková, D. Thiebaut, P. Jandera, V. Pichon, J. Sep. Sci. 32 (2009) 1036.
[23] F. Chapuis, V. Pichon, F. Lanza, B. Sellergren, M.C. Hennion, J. Chromatogr. B 804 (2004) 93.
[24] N.W. Turner, E.V. Piletska, K. Karim, M. Whitcombe, M. Malecha, N. Magan, C. Baggiani, S.A. Piletsky, Biosens. Bioelectron. 20 (2004) 1060.
[25] C. Baggiani, G. Giraudi, A. Vanni, Bioseparation 10 (2001) 389.
[26] J. Jodlbauer, N.M. Maier, W. Lindner, J. Chromatogr. A 945 (2002) 45.
[27] N.M. Maier, G. Buttinger, S. Welhartizki, E. Gavioli, W. Lindner, J. Chromatogr. B 804 (2004) 103.
[28] Z.-J. Tsao, Y.-C. Liao, B.-H. Liu, C.-C. Su, F.-Y. Yu, J. Agric. Food Chem. 55 (2007) 4921.
[29] Y. Iwahashi, H. Hosoda, J.-H. Park, J.-H. Lee, Y. Suzuki, E. Kitagawa, S.M. Murata, N.-S. Jwa, M.-B. Gu, H. Iwahashi, J. Agric. Food Chem. 54 (2006) 1936.
[30] V. Pichon, J. Chromatogr. A 1152 (2007) 41.
[31] S. Vo Duy, I. Lefebvre-Tournier, V. Pichon, F. Hugon-Chapuis, J.Y. Puy, C. Périgaud, J. Chromatogr. B 877 (2009) 1101.
[32] F. Chapuis, V. Pichon, F. Lanza, S. Sellergren, M.C. Hennion, J. Chromatogr. A 999 (2003) 23.