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1 **Peptide-based affinity media for solid-phase extraction of Ochratoxin A from wine**
2 **samples: effect of the solid support on binding properties**

3
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8
9 **Abstract.** A suitable sample clean up is a key point in the development of an analytical
10 method. Peptide-based affinity media have recently gained attention in the selective
11 extraction of defined target analytes from complex samples. In this paper we investigated the
12 thermodynamic and kinetic binding properties of different stationary phases (Amberlite IRC-
13 50, Lewatit CNP105, Toyopearl CM-650M, porous silica gel beads and micrometric glass
14 beads) functionalized with a hexapeptide sequence binding the Ochratoxin A. The highest
15 values of the equilibrium binding constant (K_{eq}) and binding site concentration (B_{max}) were
16 obtained for Lewatit CNP105 (K_{eq} : $98.1 \times 10^6 \text{ M}^{-1}$, B_{max} : $30.8 \text{ } \mu\text{mol/g}$), followed by Toyopearl
17 and micrometric glass beads, whereas the worst performances were obtained with Amberlite
18 IRC-50 and porous silica gel beads. Also kinetic performances show the same trend. These
19 results highlight that the surface chemical nature has a key role in the binding properties of
20 solid supports used as affinity media for the selective extraction of well-defined target
21 molecules. Finally, Lewatit CNP105 was compared with Amberlite IRC-50 as solid support in
22 SPE extraction of OTA from 14 wine samples fortified with OTA at 2 and $4 \text{ } \mu\text{g l}^{-1}$ levels. The
23 extracts were analyzed by HPLC with fluorescence detection (λ_{exc} 333 nm, λ_{em} 460 nm)
24 showing no significant matrix effects, **with a LOD and LOQ of 0.45 and $1.5 \text{ } \mu\text{g l}^{-1}$, respectively,**
25 and good recoveries between 71% and 108% for Amberlite IRC-50 and 91% and 101% for
26 Lewatit CNP105. While both supports showed a statistically comparable extraction accuracy,
27 a statistically significant difference was found in terms of extraction precision, confirming that
28 the solid phase based on Lewatit CNP105 performs better than the solid phase based on
29 Amberlite IRC-50.

30
31 **Keywords:** Ochratoxin A; binding peptide; binding properties; affinity chromatography; wine

1 analysis; solid-phase extraction

1. INTRODUCTION

Sample preparation is still considered the bottleneck of the whole analytical process because it can affect the unequivocal identification, confirmation and quantification of analytes. Among modern extraction techniques, immunoaffinity chromatography is one of the best ones currently able to address key issues in sample preparation: it eliminates complex and time-consuming extraction steps, increases selectivity and simplifies the experimental protocols performed on complex samples.[1] Nevertheless, immunoaffinity-based extraction methods suffer from several drawbacks as antibodies are rather expensive, show lot-to-lot variations in binding properties and are subjected to chemical degradation. All these factors have contributed to the widespread opinion that synthetic systems, mimicking the recognition properties of the antibodies, could be an alternative. Therefore, over the last few decades, many efforts are being underway to replace antibodies with synthetic ligands provided with selectivity, high load capacity, chemical resistance and cost-effectiveness. [2-5]

Peptides binding specific targets and selected by screening of combinatorial libraries have shown great potential as antibody-mimicking affinity ligands because of their versatile chemical and physical properties in addition to well-known synthetic approaches.[6-7] In the past, our research group has developed oligopeptide sequences with binding properties towards targets as estrogens [8], mycotoxins [9,10] and recombinant proteins [11]. Their high binding capacity and selectivity were obtained through a novel evolutionary combinatorial approach, based on the selection of the best sequence extracted from a starting dipeptide library screened for the binding towards a well-defined target. The best dipeptide sequence was then used as a scaffold to generate a tetrapeptide library; this iterative process of synthesis-screening-selection was continued until the binding properties showed to improve at each cycle. The selected sequences synthesized on Amberlite IRC-50 were then used as receptors in solid phase extraction protocols with performance quite comparable with those reported for commercial immunoaffinity columns, thus demonstrating that these affinity materials can replace the conventional techniques.[10]

It is well known that the analytical performance of affinity-based solid phase extraction are strictly related to the binding properties of the receptor, to its surface density and to the accessibility of the target analyte to the binding sites.[1] Therefore, the morphological features of the solid support used as scaffold should be considered a key issue in the planning of affinity media.

1 In this paper an Ochratoxin A-binding hexapeptide is synthesized on different commercial solid
2 supports based on silica or organic polymers. The chosen stationary phases are Amberlite
3 IRC50, Lewatit CNP105, Toyopearl CM-650M, porous silica gel beads and micrometric glass
4 beads. All these solid supports are commercially available at low cost and have been chosen
5 for a comparative study in view of an extended application of peptide-based solid phase
6 extraction on real samples. In fact, despite the satisfying results obtained in our previous
7 works, Amberlite IRC50 showed poor wettability, often generating erratic results on real
8 samples. From this, the choice to try other commercial solid supports with different features in
9 terms of porosity and chemical structure in order to improve analytical performance. The
10 thermodynamic and kinetic binding properties are measured to find out the peptide-based
11 solid support with the best binding features. Finally, the chosen affinity material is tested in the
12 solid phase extraction of OTA from wine samples.

13

14 **2. EXPERIMENTAL**

15 **2.1. Materials.** All the reagent were of analytical grade. Acetone, acetonitrile, methanol,
16 toluene, 4-aminobutyric acid, citric acid, sodium dihydrogen phosphate monohydrate, **sodium**
17 **hydrogencarbonate**, polyethylene glycol 8000, L-asparagine, hydrochloric acid (37% v/v), L-
18 histidine, potassium hydroxide, sodium hydroxide, sulphuric acid (96% v/v), *p*-toluenesulfonic
19 acid monohydrate were from VWR (Milano, Italy). N,N'-diisopropylcarbodiimide, N,N-
20 dimethylformamide, ethanolamine, hexamethyldisilazane, N-hydroxysuccinimide, L-leucine, ,
21 L-lysine, L-proline, L-serine, 4-(trimethoxysilyl)-butyronitrile, Amberlite IRC-50, Lewatit
22 CNP105, porous silica gel beads and micrometric glass beads were from Sigma (Milano,
23 Italy). Toyopearl CM-650M was from Tosoh HAAS (Tokyo, Japan). Ochratoxin A (OTA)
24 standard solution at a concentration of 10 µg/mL in acetonitrile (standard Oekanal) was from
25 Sigma; it was stored at -20 °C until use. Water was ultra-purified in Purelab Prima System
26 from Elga (Marlow, UK). The 0.22 µm nylon and cellulose membranes for filtration were from
27 Alltech Italia (Milano, Italy). Blank wine samples certified to be free from OTA contamination
28 were kindly provided by Neutron SpA (Modena, Italy).

29 LaChrom Elite HPLC system (programmable binary pump L-2130, autosampler L-2200, UV
30 detector L-2400, fluorescence detector L-2480) provided with EZChrom Elite Software was
31 from Merck-Hitachi (Milano, Italy).

32 **2.2. Silanization procedure.** 5.0 g of porous silica gel beads were sonicated in 50 ml of 6 M

1 hydrochloric acid, refluxed overnight, diluted with 450 ml of cold water and filtered. Then,
2 silica was washed with water till neutrality, dried overnight at 105 °C and dispersed in 100 ml
3 of toluene. After removing of water by Dean Stark apparatus and cooling at room
4 temperature, a catalytic amount of *p*-toluene sulfonic acid and 0.95 ml of 4-(trimethoxysilyl)-
5 butyronitrile (5 mmoles) were added and the dispersion refluxed for 3 hours. The silanized
6 silica was filtered, washed with toluene, and dispersed in 100 ml of fresh toluene. After the
7 removal of water and the cooling as above, 0.81 ml of hexamethyldisilazane (5 mmoles) were
8 added and the silica dispersion let to react overnight under gentle agitation to block any
9 remaining silanolic group. The end-capped silica was filtered and washed repeatedly with
10 small amounts of toluene and acetone. Then, it was transferred in 40 ml of a solution of
11 sulfuric acid-water (1:1 v/v) to obtain the hydrolysis of nitrile to carboxylic acid, refluxed for 1
12 hour and then, after cooling, filtered and neutralized with several washes of water and
13 acetone. Glass beads were treated in the same aforementioned manner after a preliminary
14 etching step to increase the surface available for the peptide synthesis. For this purpose, a
15 solution of 6 M potassium hydroxide was added to the glass beads, the dispersion boiled for
16 24 hours and the beads washed with a plenty amount of water to achieve the neutrality.

17 **2.3. Solid-phase peptide synthesis.** All the solid phases were acidified with 1 M
18 hydrochloric acid, neutralized with water and dried in acetone. 4-aminobutyric acid was
19 introduced as spacer arm by reaction with the available carboxylic functions (estimated by
20 back-titration) through the *N*-hydroxysuccinimide/carbodiimide method.[12] The OTA-binding
21 hexapeptide Ser-Asn-Leu-His-Pro-Lys was synthesized on the surface of each solid phase as
22 previously described in literature.[10] Briefly, the carboxyl groups of the spacer arm grafted
23 onto the beads were activated for 2 h in *N,N*-dimethylformamide with *N*-
24 hydroxysuccinimide/carbodiimide, then the first amino acid of the peptide sequence was
25 dissolved in sodium hydrogencarbonate buffer (0.15 M, pH 8.5) and added in 3:1 excess to
26 the available carboxyls. After a 24 h reaction, the beads were washed with *N,N'*-
27 dimethylformamide and the protocol was repeated to add a new amino acid in the sequence.

28 **2.5. Reverse-phase liquid chromatography.** Reversed-phase HPLC with fluorescence
29 detection (excitation wavelength at 333 nm and emission wavelength at 460 nm) was used to
30 measure OTA. The analytical column was a 100 x 4.6 mm, 5 μm, LiChrospher 100 RP-18
31 from VWR (Milano, Italy). The mobile phase was composed of acetonitrile–water–acetic acid
32 (55:44:1 v/v/v) and the elutions were performed under isocratic conditions at a flow rate of 0.5

1 ml/min. The sample volume injected was 10 μl . OTA retention time was 3.84 min. Reference
2 standard solutions of OTA at concentrations of 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10, 25, 50 and
3 100 $\mu\text{g l}^{-1}$ were analyzed three times consecutively and peak areas were plotted against
4 concentration. A calibration plot was drawn by using weighted linear regression (weight =
5 1/conc). ~~Calculated LOQ was 1.5 $\mu\text{g l}^{-1}$~~ Calculated LOD and LOQ were 0.45 and 1.5 $\mu\text{g l}^{-1}$,
6 respectively.

7 **2.6. Study of binding isotherms.** 2 ml of OTA solution at concentrations of 100, 50, 20, 10,
8 5, 2 and 1 $\mu\text{g l}^{-1}$ in a 0.1 M phosphate-citrate buffer pH 4.0 were added to 20 mg of solid
9 phase into 3 ml-amber glass vials. The solutions were sonicated for 5 minutes and incubated
10 overnight at room temperature on a horizontal shaking device. Then, the solutions were
11 filtered and the free amount of Ochratoxin A was measured by HPLC analysis. Each
12 experimental point of the binding isotherm was assessed as the average of three repeated
13 measures. The binding isotherms were built by using Table Curve 2D 5.0 (Systat Software
14 Inc., Richmond, CA, USA). Non-linear least square fitting was applied to the averaged
15 experimental data by using the Langmuir isotherm equation:

$$16$$
$$17 \quad B = B_{\max} K_{\text{eq}} F / (1 + K_{\text{eq}} F)$$
$$18$$

19 where B is the OTA bound to the solid phase, F is the OTA not bound to the polymer, K_{eq} is
20 the equilibrium binding constant and B_{\max} the binding site density. To obtain robust results,
21 Pearson VII limit minimization was chosen as minimization method. The fitting was carried out
22 several times by using different initial guess values for the isotherm parameters to avoid being
23 trapped in local minima which would give incorrect results.

24 **2.7. Study of binding kinetics**

25 1 ml of a 40 $\mu\text{g l}^{-1}$ OTA solution in 0.1 M phosphate-citrate buffer pH 4.0 was added to 20 mg
26 of solid phase previously suspended in 1 ml of the same buffer. The vials were vortexed and
27 let to equilibrate at room temperature in a horizontal shaking device for 1, 2, 3, 5, 10, 15 and
28 20 minutes. Therefore, the solutions were filtered and the free amount of OTA was measured
29 by HPLC analysis. Each partition experiment was repeated three times and the amounts of
30 free OTA were evaluated as averaged values. The kinetic curves ~~reported in figure 2~~ were
31 built by using Table Curve 2D 5.0. Non-linear least square fitting was applied to the averaged

1 experimental data by using a 1st order kinetic equation:

2

$$3 B_t = B_{eq} [1 - \exp(-k_a t)]$$

4

5 where B_t is the amount of OTA bound to the solid phase at the t time, B_{eq} is the amount of
6 OTA bound to the solid phase at the equilibrium and k_a is the association rate constant.

7 **2.8 Peptide-solid phase extraction of OTA from wine samples**

8 The followed extraction protocol has been already described [10]. Aliquots of about 500 mg of
9 peptide-based solid support were suspended in phosphate-citrate buffer (20 mM, pH 4.0),
10 sonicated in a water-bath for 10 min and packed in 5-ml empty polypropylene SPE cartridges
11 provided with frits to secure the packing and outlet stopcocks. The columns were connected
12 to a vacuum manifold, washed with 3x1ml of water-methanol (3:7 v/v) and equilibrated with
13 3x1ml of phosphate-citrate buffer. Blank wine samples were fortified with 2.0 and 4.0 $\mu\text{g l}^{-1}$ of
14 OTA and, to precipitate tannins, samples were diluted 1:1 (v/v) with a 1% (v/v) aqueous
15 solution of polyethylene glycol 8000, incubated at 4°C overnight, centrifuged at 8000 rpm for
16 15 min and filtered on 0.22 μm cellulose membranes. Then, 1 ml of sample was loaded, and a
17 vacuum was applied to facilitate the passage of the sample through the cartridge bed. After
18 sample loading, the cartridges were sequentially washed with 500 μl of phosphate-citrate
19 buffer and 500 μl of acetonitrile. OTA was recovered by eluting the columns with ~~an~~ additional
20 ~~3x~~500 μl of acetonitrile. To evaluate the reproducibility of the extraction protocol, each
21 extraction was repeated five times and OTA recovery was evaluated as the average of the
22 single values measured.

23

24 **3. RESULTS AND DISCUSSION**

25 In a previously published paper, we have described the preparation and the application of a
26 OTA-binding hexapeptide supported on Amberlite IRC-50 to selectively extract the mycotoxin
27 from wine samples.[10] The binding performances of this affinity system confirms that
28 peptide-based solid phase extraction may be considered a viable alternative to conventional
29 immunoaffinity chromatography. Amberlite IRC-50 has been used as solid support by our
30 group for developing different applications of peptide-based solid phase extraction because of
31 its commercial availability and surface chemical properties. However, the extensive use of this

1 support on real samples often showed erratic analytical results, mainly due to its poor
2 wettability. Of consequence, in this paper we investigated other solid supports commercially
3 available as an alternative to Amberlite IRC-50. A stationary phase for affinity solid phase
4 extraction should be cheap, with good hydrophilic properties for application in polar media,
5 provided with reactive surface functional groups and with good surface accessibility. These
6 features allowed us to choose as possible candidates Lewatit CNP105, Toyopearl CM-650M,
7 porous silica gel beads and micrometric glass beads. As reported in table 1, the chosen solid
8 phases showed very different features in terms of chemical composition (polymethacrylate,
9 silica, borosilicate glass), bead dimensions (ranging from about 10 to 700 μm), porosity (non
10 porous, meso- and macroporous) and surface area (from 0.1 to 500 m^2/g). It should be noted
11 that the only property common to all these solid phases is the surface chemistry, due to the
12 presence of carboxyl groups useful for the covalent attachment of the binding peptide
13 sequences. Therefore, the experimental work started with the study of the binding properties
14 of chosen hexapeptide-based solid phases in order to investigate their binding ability in
15 function of their different morphological and structural features.

16 **3.1. Study of the binding isotherms**

17 Figure 1 shows the different binding isotherms obtained, whereas table 2 summarized the
18 measured binding properties of the different solid phases. In detail, B_{max} represents the
19 binding site concentration accessible to OTA measured in conditions of saturation, K_{eq}
20 represents the equilibrium binding constant, and the product $B_{\text{max}}K_{\text{eq}}$ estimates the overall
21 binding capacity of the solid phases related both to the binding site concentration and to the
22 equilibrium binding constant. A comparison between table 1 and table 2 shows the absence
23 of a direct relationship between the values of K_{eq} and structural features of the stationary
24 phases as porosity and surface area. All the values obtained range from about 10 to 98×10^6
25 M^{-1} , then within an order of magnitude despite the high range of variation of the morphological
26 features of the tested solid phases. The trend of B_{max} values seems instead related to the
27 carboxylic groups available on the surface with the exception of Amberlite IRC-50 which
28 shows a B_{max} value sharply lower with respect to that expected on the basis of the amounts of
29 the surface carboxylic groups. This phenomenon could be due to the high hydrophobicity of
30 Amberlite IRC-50 that reduces the surface wettability compromising the reactivity of the solid
31 phase, and bringing to an increase of mismatched peptide sequences with a severe drop of
32 the affinity for the mycotoxin.

1 Porous silica gel and glass beads show comparable concentrations of the carboxylic groups
2 and similar B_{max} values despite a great difference in surface area. As glass beads are non
3 porous material, binding sites are all placed on the surface, while the same is not true for
4 porous silica gel. Thus, to explain comparable values for B_{max} , it should be assumed that
5 glass beads present a great surface roughness. This is plausible because of the preliminary
6 etching step with 6 M potassium hydroxide performed onto glass beads as supported by
7 literature.[13]

8 A further parameter to estimate the binding behavior can be calculated as the percent ratio
9 between the binding site concentration (i.e. B_{max}) and the available carboxylic groups. The
10 values obtained are: 0.45% for Toyopearl CM-650M, 0.47% for Lewatit CNP105, 0.45% for
11 glass beads, 0.57% for silica gel, and 0.064% for Amberlite IRC-50. This result again confirms
12 the poorly binding properties of Amberlite IRC-50 with respect to the other supports. This
13 point out that data of porosity and surface area do not explain the overall binding properties of
14 the stationary phases. Moreover, it is worth observing that the calculated percentage are very
15 low in any case and this means that either the resulting yield of functionalization is markedly
16 reduced or only a reduced amount of peptide sequences are available for the binding.

17 The trend of the overall binding capacity ($B_{max}K_{eq}$) seems to be mainly affected by the B_{max}
18 value (i.e. the binding site concentration) rather than from the binding constant as shown in
19 table 2.

20 **3.2. Study of the binding kinetics**

21 Figure 2 shows the different binding kinetics obtained, whereas table 3 shows the calculated
22 values of the total binding site concentration B_{eq} , the association rate constant (k_a), the half-
23 life time $t_{1/2}$ and the related statistical parameters of the fit. The B_{eq} values decrease in the
24 order Lewatit CNP105 > Toyopearl CM-650M > glass beads > silica gel > Amberlite IRC-50,
25 proving that the mycotoxin amount bound at a defined time follows the same trend observed
26 in the thermodynamic studies. Moreover, the association constant k_a shows high values in the
27 case of glass beads and Toyopearl CM-650M which are respectively a non porous and a
28 macroporous stationary phase. These features assure high association rates because of the
29 ease of access to the binding sites that are – as previously stated – well exposed to the
30 surface. Amberlite IRC-50 shows the lowest value of k_a confirming that the hydrophobic
31 feature strongly slows the entry into the binding sites.

32 **3.3 Peptide-based solid phase extraction OTA from wine samples**

1 The thermodynamic and kinetic binding studies allowed us to confirm that Amberlite IRC-50
2 shows limited binding properties whereas, among the tested stationary phases, Lewatit
3 CNP105 seems to be the best one in terms of thermodynamic and kinetic binding constants
4 and binding site concentration. So, in view of using Lewatit CNP105 as solid support in
5 peptide-based solid phase extraction, we performed recovery tests of OTA from blank wine
6 samples spiked with known amounts of mycotoxin. The analysis was performed according to
7 literature and used to prove the applicability of these materials for selective analyte
8 extraction.[14]

9 The recovery of OTA was determined by comparing the detector response of 14 different wine
10 samples. Recoveries reported in Table 3 were determined at two concentration levels of OTA
11 (2.00 and $4.00 \mu\text{g l}^{-1}$) and came out at levels between about 71% and 108% on Amberlite
12 IRC-50 and about 91% and 101% on Lewatit CNP105. A t-test performed to compare the
13 recovery obtained at both OTA concentrations showed that the difference in the mean values
14 between the two groups is not great enough to exclude the possibility that the difference is
15 due to random sampling variability (mean recovery at $2.00 \mu\text{g l}^{-1}$: $89.8 \pm 9.2\%$ for Amberlite
16 IRC-50, $94.5 \pm 3.1\%$ for Lewatit CNP105, $t=1.800$, $P=0.0835$; mean recovery at $4.00 \mu\text{g l}^{-1}$:
17 $92.1 \pm 9.3\%$ for Amberlite IRC-50, $96.2 \pm 2.9\%$ for Lewatit CNP105, $t=1.583$, $P=0.126$); thus
18 there is not a statistically significant difference in terms of extraction accuracy. On the
19 contrary, a F-test performed to compare the recovery variances calculated for 14 wine
20 samples at both OTA concentrations showed that the differences in the recovery variances
21 are great enough to be considered statistically different (mean variance at $2.00 \mu\text{g l}^{-1}$: 265.9
22 for Amberlite IRC-50, 32.3 for Lewatit CNP105, $F=8.230$; mean variance at $4.00 \mu\text{g l}^{-1}$: 259.6
23 for Amberlite IRC-50, 31.1 for Lewatit CNP105, $F=8.351$); thus there is a statistically
24 significant difference in terms of extraction precision confirming that the solid phase based on
25 Lewatit CNP105 performed better than the solid phase based on Amberlite IRC-50 .

26

27 **4. CONCLUSIONS**

28 A Ochratoxin A-binding hexapeptide was synthesized on different commercial solid supports to
29 be used as peptide-based affinity media for the selective extraction of OTA according to
30 previously described approach. The chosen stationary phases were Amberlite IRC50, Lewatit
31 CNP105, Toyopearl CM-650M, porous silica gel beads and micrometric glass beads, all

1 commercially available. The study of their thermodynamic and kinetic binding properties was
2 accomplished in order to choose the solid support more appropriate for solid phase
3 extraction. The experimental results showed that properties such as surface area, porosity
4 and available surface carboxylic groups have less importance with respect to the chemical
5 composition of the stationary phases. Lewatit CNP105 showed to be the one with the most
6 appropriate binding features, followed by Toyopearl CM-650M, and then by glass beads and
7 silica gel whose binding properties not so different despite the great difference in structure.
8 The worst properties were indeed shown by Amberlite IRC-50. Peptide-base solid phase
9 extractions of OTA from spiked wine samples were successfully performed with Lewatit
10 CNP105 as solid support. Extraction results showed a better precision if compared with those
11 obtained on Amberlite IRC-50.

1 **BIBLIOGRAPHY**

- 2 [1] V.Pichon, M.Delaunay-Bertoncini, M.B.Hennion, Immunosorbents in sample preparation,
3 in: J. Pawliszyn (Ed.) Sampling and sample preparation for field and laboratory
4 (Comprehensive Analytical Chemistry Ser., Vol. 37), Elsevier Science, Amsterdam,
5 2002, pp. 1081-1100.
- 6 [2] D.Hutanu, V.T.Remcho. Aptamers as molecular recognition elements in chromatographic
7 separations, in: E.Grushka, N.Grinberg (Eds.) Advances in Chromatography, Vol.45,
8 CRC Press, Boca Raton, USA, 2007, pp.173-196.
- 9 [3] B.T.S.Bui, K.Haupt, Molecularly imprinted polymers: synthetic receptors in bioanalysis.
10 Anal.Bioanal.Chem., 398 (2010) 2481-2492.
- 11 [4] E.Turiel, A.Martin-Esteban, Molecularly imprinted polymers for sample preparation: A
12 review. Anal.Chim.Acta, 668 (2010) 87-99.
- 13 [5] V.Pichon, F.Brothier, A.Combes, Aptamer-based-sorbents for sample treatment. A
14 review. Anal.Bioanal.Chem., 407 (2015) 681-698.
- 15 [6] C.Tozzi, G.Giraudi, Antibody-like peptides as a novel purification tool for drugs design.
16 Curr. Pharm.Design., 12 (2006) 191-203.
- 17 [7] S.Pavan, F.Berti. Short peptides as biosensor transducers. Anal.Bioanal.Chem., 402
18 (2012) 3055-3070.
- 19 [8] C.Tozzi, L.Anfossi, G.Giraudi, C.Giovannoli, C.Baggiani, A.Vanni, Chromatographic
20 characterisation of an estrogen-binding affinity column containing tetrapeptides selected
21 by a combinatorial-binding approach. J.Chromatogr.A, 966 (2002) 71-79.
- 22 [9] C.Tozzi, L.Anfossi, C.Baggiani, C.Giovannoli, G.Giraudi. A combinatorial approach to
23 obtain affinity media with binding properties towards the aflatoxins. Anal.Bioanal.Chem.,
24 375 (2003) 994-999.
- 25 [10] G.Giraudi, L.Anfossi, C.Baggiani, C.Giovannoli, C.Tozzi. Solid-phase extraction of
26 Ochratoxin A from wine based on a binding hexapeptide prepared by combinatorial
27 synthesis. J.Chromatogr.A, 1175 (2007) 174-180.
- 28 [11] C.Tozzi, L.Anfossi, C.Baggiani, C.Giovannoli, G.Giraudi. Synthetic peptides as artificial
29 receptors towards proteins from genetically modified organisms. Biosens.Bioelectron.,
30 24 (2008) 493-497.
- 31 [12] G.W.Anderson, J.E.Zimmerman, F.M.Callahan. N-Hydroxysuccinimide esters in peptide
32 synthesis. J.Am.Chem.Soc., 85 (1963) 3039-3039.

1 [13] Q.Zhang, T.Zhang, J.Ge, Y.Yin. Permeable silica shell through surface-protected
2 etching. Nano Letters 8 (2008) 2867-2871.

3 [14] P.Ding, Z.Mi, Y.Hou, Y.He, J.Xie. Determination of Ochratoxin A in feed by
4 immunoaffinity cleanup and liquid chromatography, J.AOAC Int., 96 (2013) 599-602.

5

1 **TABLES**

2

3 **Table 1:** features of solid phases considered

4

Name	Type	Bead diameter, μm	Porosity, nm	Surface area, m^2/g	Available COOH, mmoles/g
Amberlite IRC-50	4% DVB cross-linked polymethacrylate	280-700	35-40	500	10.67
Lewatit CNP105	polymethacrylate	100-400	27	200	6.60
Silica gel	silica	35-45	8-12	260-340	0.87
Glass beads	borosilicate glass	9-13	none	0.10-0.15	1.10
Toyopearl CM-650M	hydroxylated polymethacrylate	65	1000	20	1.40

5

6

7 **Table 2:** isotherm parameters for OTA binding on considered solid phases. B_{max} : binding site concentration; K_{eq} : equilibrium binding constant; $B_{\text{max}}K_{\text{eq}}$: binding capacity; r^2 : Pearson's correlation coefficient; SEE: fit standard error

10

Name	B_{max} , $\mu\text{mol/g}$	K_{eq} , M^{-1} , $\times 10^{-6}$	r^2	SEE	$B_{\text{max}}K_{\text{eq}}$
Amberlite IRC-50	6.81 \pm 1.1 (t=6.28, P=0.00150)	10.2 \pm 3.5 (t=2.93, P=0.03245)	0.9746	0.316	69
Lewatit CNP105	30.8 \pm 4.7 (t=7.03, P=0.00593)	98.1 \pm 33 (t=2.97, P=0.05914)	0.9839	1.289	3020
Silica gel	4.93 \pm 0.31 (t=16.13, P=0.00009)	13.1 \pm 1.5 (t=9.03, P=0.00089)	0.9978	0.054	65
Glass beads	4.97 \pm 0.80 (t=6.25, P=0.00154)	50.8 \pm 26 (t=2.59, P=0.04858)	0.9037	1.975	252
Toyopearl CM-650M	15.7 \pm 0.54 (t=29.24, P<0.00001)	30.4 \pm 2.8 (t=10.94, P=0.00011)	0.9975	0.254	477

11

1 **Table 3:** kinetic parameters for OTA binding on considered solid phases. B_{eq} : binding site
 2 concentration; k_a : association rate constant; $t_{1/2}$: half-life time; r^2 : Pearson's correlation
 3 coefficient; SEE: fit standard error

4

Name	B_{eq}, μM	k_a, min^{-1}	$t_{1/2}$, min	r^2	SEE
Amberlite IRC-50	1.06±0.2 (t=5.22, P=0.00122)	0.0758±0.025 (t=3.09, P=0.01768)	9.14±3.0	0.9548	0.0648
Lewatit CNP105	5.08±0.13 (t=38.66, P<0.00001)	0.340±0.029 (t=11.69, P=0.00001)	2.94±0.25	0.9880	0.2153
Silica gel	1.44±0.014 (t=103.89, P<0.00001)	0.560±0.022 (t=25.50, P<0.00001)	1.79±0.070	0.9973	0.0276
Glass beads	1.90±0.015 (t=128.51, P<0.00001)	0.811±0.031 (t=26.34, P<0.00001)	1.23±0.032	0.9977	0.0325
Toyopearl CM-650M	3.59±0.093 (t=38.52, P<0.00001)	0.838±0.11 (t=7.76, P=0.00011)	1.19±0.15	0.9745	0.2058

5

1 **Table 4:** OTA extraction recovery \pm 1 standard deviation obtained at concentration levels of
 2 2.00 and 4.00 $\mu\text{g l}^{-1}$ in 14 different wines. Each recovery was calculated as the average of
 3 five repeated measures.

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Wine sample	peptide on Amberlite IRC-50		peptide on Lewatit CNP105	
	recovery (%) (2.00 $\mu\text{g l}^{-1}$)	recovery (%) (4.00 $\mu\text{g l}^{-1}$)	recovery (%) (2.00 $\mu\text{g l}^{-1}$)	recovery (%) (4.00 $\mu\text{g l}^{-1}$)
Arneis	100 \pm 9.5	83.7 \pm 13	98.8 \pm 3.5	94.8 \pm 4.8
Asti Spumante	89.2 \pm 13	108 \pm 16	94.2 \pm 5.3	100 \pm 6.1
Moscato d'Asti	84.9 \pm 17	91.6 \pm 17	95.5 \pm 5.5	95.2 \pm 5.2
Montepulciano d'Abruzzo	94.2 \pm 12	85.6 \pm 13	91.4 \pm 3.4	94.8 \pm 4.2
Rosato di Schiacca	78.3 \pm 16	77.1 \pm 13	93.1 \pm 4.9	90.5 \pm 3.8
Malvasia di Casorzo	71.1 \pm 15	90.5 \pm 13	88.6 \pm 5.5	94.6 \pm 5.1
Marsala	103 \pm 14	98.1 \pm 14	97.4 \pm 5.2	95.9 \pm 5.4
Barbera	85.9 \pm 22	85.2 \pm 16	92.1 \pm 7.4	95.3 \pm 6.1
Barbera d'Asti	99.8 \pm 21	100 \pm 19	95.8 \pm 7.3	101 \pm 6.7
Dolcetto d'Alba	90.4 \pm 14	95.2 \pm 16	92.1 \pm 5.5	96.2 \pm 6.2
Dolcetto di Langa	85.0 \pm 14	103 \pm 16	94.5 \pm 5.3	101 \pm 6.2
Merlot	91.9 \pm 11	102 \pm 12	95.6 \pm 4.6	94.2 \pm 4.1
Nebbiolo	100 \pm 13	90.0 \pm 13	100 \pm 4.6	96.9 \pm 4.7
Pelaverga	83.1 \pm 14	79.4 \pm 14	93.3 \pm 5.1	96.0 \pm 5.0

5

1 **LEGEND OF FIGURES**

2

3 **Figure 1:** OTA binding isotherms for different stationary phases functionalized with the OTA-
4 binding hexapeptide Ser-Asn-Leu-His-Pro-Lys. Open circles: Amberlite IRC-50; gray circles:
5 Lewatit CNP105; red circles: Toyopearl CM-650M; blue circles: silica gel; green circles: glass
6 beads.

7

8 **Figure 2:** OTA binding kinetics for different stationary phases functionalized with the OTA-
9 binding hexapeptide Ser-Asn-Leu-His-Pro-Lys. Open circles: Amberlite IRC-50; gray circles:
10 Lewatit CNP105; red circles: Toyopearl CM-650M; blue circles: silica gel; green circles: glass
11 beads.