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

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Abstract. A suitable sample clean up is a key point in the development of an analytical method. Peptide-based affinity media have recently gained attention in the selective extraction of defined target analytes from complex samples. In this paper we investigated the thermodynamic and kinetic binding properties of different stationary phases (Amberlite IRC-50, Lewatit CNP105, Toyopearl CM-650M, porous silica gel beads and micrometric glass beads) functionalized with a hexapeptide sequence binding the Ochratoxin A. The highest values of the equilibrium binding constant (K_{eq}) and binding site concentration (B_{max}) were obtained for Lewatit CNP105 (K_{eq}: 98.1x10^6 M^{-1}, B_{max}: 30.8 μmol/g), followed by Toyopearl and micrometric glass beads, whereas the worst performances were obtained with Amberlite IRC-50 and porous silica gel beads. Also kinetic performances show the same trend. These results highlight that the surface chemical nature has a key role in the binding properties of solid supports used as affinity media for the selective extraction of well-defined target molecules. Finally, Lewatit CNP105 was compared with Amberlite IRC-50 as solid support in SPE extraction of OTA from 14 wine samples fortified with OTA at 2 and 4 μg l^{-1} levels. The extracts were analyzed by HPLC with fluorescence detection (λ_{exc} 333 nm, λ_{em} 460 nm) showing no significant matrix effects, with a LOD and LOQ of 0.45 and 1.5 μg l^{-1}, respectively, and good recoveries between 71% and 108% for Amberlite IRC-50 and 91% and 101% for Lewatit CNP105. While both supports showed a statistically comparable extraction accuracy, a statistically significant difference was found in terms of extraction precision, confirming that the solid phase based on Lewatit CNP105 performs better than the solid phase based on Amberlite IRC-50.

Keywords: Ochratoxin A; binding peptide; binding properties; affinity chromatography; wine

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 synthetized 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.

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

2. EXPERIMENTAL

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

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

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

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

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

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

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

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

$$B= B_{max}K_{eq}F/(1+K_{eq}F)$$

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

2.7. Study of binding kinetics

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

experimental data by using a 1st order kinetic equation:

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

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

2.8 Peptide-solid phase extraction of OTA from wine samples

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

3. RESULTS AND DISCUSSION

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

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

3.1. Study of the binding isotherms

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

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

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

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

3.2. Study of the binding kinetics

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

3.3 Peptide-based solid phase extraction OTA from wine samples

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

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

4. CONCLUSIONS

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

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

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TABLES

Table 1: features of solid phases considered

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

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

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

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

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

Table 4: OTA extraction recovery ± 1 standard deviation obtained at concentration levels of 2.00 and 4.00 μg l^{-1} in 14 different wines. Each recovery was calculated as the average of five repeated measures.

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

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

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