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Short communication

Bio-inspired solid phase extraction sorbent material for cocaine: A cross reactivity study

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ABSTRACT

The binding specificity of a bio-inspired hexapeptide (QHWWDW) versus cocaine and four other drugs such as 3,4-methylenedioxy-N-methylamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), phencyclidine and morphine was computationally studied and then experimentally confirmed in solid phase extraction (SPE) followed by liquid chromatography–mass spectrometry (LC/MS) detection. In simulation, the hexapeptide-drug complexes were docked with different scoring functions and considering pH chemical environment. In experimental, the cross reactivity of the selected hexapeptide was tested as SPE sorbent versus cocaine and other four drugs using buffer solutions at pH 4 and 7. Significant differences in specific retention were found between cocaine (97% of recovery) and both morphine (45% of recovery) and phencyclidine (60% of recovery), but less for ecstasies (average recovery 69%). In agreement with docking simulation, the hexapeptide showed the highest recovery with best specificity versus cocaine at pH 7 with an experimentally binding constant of $2.9 \times 10^6\ M^{-1}$. The bio-inspired sorbent material analytical performances were compared with a commercial reversed phase cartridge confirming the hexapeptide specificity to cocaine and validating simulated data.

1. Introduction

Cocaine is a well-known sympathomimetic drug representing a considerable health-emergency for publics [1,2]. This illicit drug and its metabolites are more and more considered as the latest group of emerging environmental pollutants [3,4] and the identification of non-approved drugs is a great challenge for control laboratories. The robustness of methods and techniques for identification and discrimination of illegal and legal drugs of abuse has been investigated in different works [5,6].

Analytical and bio-analytical methods were proposed for drug monitoring and detection in samples [7–9]. The most explored method is, in different experimental design configurations, the use of solid phase extraction followed by chromatography–mass spectrometry [9–13]. In general, there is a great interest in the development of pre-analytical tools for clean-up and pre-concentration of analytes [14–16]. In fact, sorbent materials are often not selective and can result in the co-elution of interfering compounds with similar polarity, affecting the reliability of the analytical methods. To overcome this issue, different approaches have been studied in order to produce specific affinity-based stationary phases. To this end, selective ligands such as molecularly imprinted polymers, aptamers or peptides have been developed, offering a viable and cost-effective alternative to antibodies which are expensive and challenging to prepare [14,17–20]. These engineered receptors are designed to target specific molecules, similarly to enzymes or biological receptors. They usually show lower affinities but they also offer some advantages such as low costs, rapid synthesis and stability.

In this work, the application of a bio-inspired molecularly modeled peptide was proved to be a selective sorbent material for cocaine vs. ecstasies, phencyclidine and morphine. The experimental data was supported by a molecular docking procedure. Over the last decade molecular docking has demonstrated its usefulness, in areas such as the identification of lead compounds and drug discovery [21–23]. This approach has been very often used in combination with the implementation of consensus scoring [24], considerations on both ligand and receptor flexibility [25], and the inclusion of semi-empirical and/or molecular mechanics methods for the assessment of the binding energies [26–29]. The simulated

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conformations of receptors when interacting with their target molecules have demonstrated their potential and predictive capabilities for subsequent development of experimental methods [20,30–35]. An important goal in molecular design is to collect several datasets for the improvement of docking and scoring methods [10,36,37]. In particular, the modification of basic parameters in molecular modeling software was shown to have a significant effect on docking and virtual screening results [38–42].

This work shows how molecular modeling method can be used as a convenient tool for the optimization of SPE conditions. The key point in optimizing experimental cocaine specificity lies on considering the effect of the pH. This was calculated here by changing protonation states of histidine. Moreover, the orientation of the complexes within the binding site, outputted by the docking software, was very useful in experimental strategy design.

The introduction of predictive computational models in analytical protocols, instead of trial and error procedures, offers advantages in minimizing experimental problems currently encountered, such as non-specific recognition, reagent stability and separation procedures.

2. Materials and methods

2.1. Virtual screening process

A desktop PC with a 3.4 GHz Intel Core I7-2600 processor, 8 GBytes DDR3 RAM with 1333 MHz bus, running Microsoft Windows 7 Professional 64 Bits was used for the entire screening process, molecular modeling experiments, post-calculations and data analysis.

The virtual screening process was carried out using an automated pipeline of computational tools bundled in OpenEye Scientific Software package under academic license. The automation was achieved with AutoIT V3, a freeware BASIC-like scripting language. A database of ligands was generated by converting standard IUPAC names into structures using LEXICHEM package [43]. Each of these structures was subsequently optimized using molecular mechanics as implemented in SZYBKI 1.5.7 in its default parameterization [44]. In order to contemplate molecules flexibility, for both receptor and ligands a set of conformers was generated with OMEGA 2.4.6 [45,46]. An exhaustive rigid body docking was implemented using FRED 2.2.5 and 3.0.0 [47]. The visualization of molecular structures for pre- and post-processing and analysis was carried out with VIDA 4.2.1 [48].

The hexapeptide QHWWDW was designed in zwitterion form with one or two NH bonds on the imidazole ring, aiming to simulate its behavior at pH 7 and pH 4-. The net charge at pH 7, its isoelectric point and hydropathy index were calculated using a peptide calculator [49].

During the docking process, the entire surface of each hexapeptide conformer was considered suitable to form positive interactions with ligand molecules. In consequence, a box, defining the docking active site, was generated for each conformer encapsulating the entire peptide, with sizes comprised from 4500 $Å^3$ to 7200 $Å^3$. The required time to process each conformer, from the initial design to final docking stage, was about 1 min.

FRED 3.0.0 was run only in its default parameterization with Chemgauss4 function, a modification of Chemgauss3 (Ch3), with improved hydrogen bonding and metal chelator terms. Instead, with FRED 2.2.5, three parameters were tested: 1-poses ranking through exhaustive scoring; 2-systematic solid body optimization functions; and 3- consensus structure score evaluation.

The default solid body optimization function in FRED 2.2.5 was Ch3, but also, Chemgauss2 (Ch2) and Shapegauss (Sh) functions were tested. Functions PLP and CGO were not considered in this work because they gave no effective poses.

The available alternatives to FRED 2.2.5 default scoring function Ch3 were Sh, PLP, CGO, CGT Ch2, Ch3, Chemscore (Cs), OEChemscore (Ocs), Screenscore (Ss), or none (Nn), each one of them presenting a particular combination of speed and atomic interactions awareness. CGO and CGT were not suitable in this type of simulation experiment, therefore, when tested, both functions generated errors and were discarded.

By default FRED 2.2.5 used a consensus of multiple scoring functions to rank one ligand against another. This consensus score was calculated based on the combined results of PLP, Ch3 and Ocs. However, different combinations of other scoring functions available (Sh, PLP, CGO, CGT Ch2, Ch3, Cs, Ocs, Ss and Zapbind) were also used in this study. Consensus scoring failed when using functions PLP, Zapbind, CGO and CGT. These errors occurred due to internal unexpected miscalculations in the software, atom types mismatch, and other factors.

The scoring function was given by the sum of different terms like shape, hydrogen bond, aromatic, desolvation and others. The major difference in scoring functions was the use or exclusion of these terms in calculating the score. None of the functions had intramolecular terms.

3. Extraction procedure and liquid chromatography–mass spectrometry analysis (LC–MS/MS)

3.1. Chemicals

Standards of cocaine (COC), 3,4-methylenedioxy-N-methylamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), phencyclidine (PCP) and morphine (MOR) were purchased from LGC Standard (Italy). The purity of the reference compounds was $\geq$ 99%. All standards were provided at a concentration of 3 mM. Individual stock solutions were prepared in methanol at 300 μM and working standard mixtures were prepared by appropriate dilution of the standard solutions in methanol. All solutions were stored at −20 °C in dark condition. Acetonitrile and methanol were of RS-Plus grade. Ultrapure water was produced by a Milli-Q Plus apparatus from Millipore (USA). Acetonitrile and methanol were of RS-Plus grade. All reagents used for the preparation of aqueous buffers, were purchased from Carlo Erba (Italy).

The solid phase extraction sorbent material QHWWDW-resin (Nova Syn TGA), with a peptide substitution level of 0.17 mmol g^{-1} was synthetized by EspiKem srl (Italy). Strata-X 33 μm polymeric reversed phase cartridges (30 mg/mL) were from Phenomenex. SPE Isolute column (Empty 1 mL Reservoir) was from STEPBIO (Italy).

3.2. Extraction procedure

The cartridges (volume 1 ml) were packed with 30 mg of resin (the blank) or modified peptide resin dissolved in 5 mL of an ethanol/water solution (80:20, v/v) and kept at room temperature for 6–8 h. This suspension was slowly loaded into the cartridge with a teflon frit on the bottom. During this procedure, the cartridge was continuously shaken in order to obtain a homogeneous packing. After loading, a second frit was used to cover the resin into the cartridge. Then the cartridge was conditioned and equilibrated by washing with ethanol. All the experiments were carried out by means of a VISIPREP device and the solvent fractions collected were named progressively.

The extraction procedure was performed in four steps:

1. Conditioning of the stationary phase with Tris–HCl (pH = 7).
2. Sample loading (1 mL).

Table 1
Selected transitions and main LC–MS/MS parameters.

Analyte	t_R (min)	Q1 (amu)	DP (V)	FP (V)	EP (V)	Q3 (amu)	CE (V)	CXP (V)
COC	4.50	304.0	26	400	8	182.2	26	5
						82.2	45	9
MDMA	2.87	194.1	18	400	6	163.2	17	5
						105.2	35	7
MDEA	3.36	208.0	18	400	6	163.2	17	6
						105.1	35	10
PCP	5.37	244.1	11	400	8	86.1	18	11
						91.1	42	11
MOR	1.72	286.0	42	400	11	201.2	35	8
						153.2	53	10

3. Washing with 1 mL of ultrapure water.
4. Elution with 1 mL of formic acid 5 mM in methanol.

The same extraction procedure was applied to cartridges packed with sorbent material QHWWDW-resin, the resin without peptide (the blank) and using Strata-X.

3.3. LC–MS/MS analysis

A Micro-LC Pump with autosampler (equipped with a 5 μL loop) and vacuum degasser, system Perkin Elmer series 200 (USA), was used for the chromatographic run. The LC system was coupled to a triple quadrupole mass spectrometer, API 2000 from PE-Sciex (Canada), equipped with a TurbolonSpray source.

The analytes were separated using a reversed phase C18 Kinetex XB column (10 cm × 2.1 mm ID) from Phenomenex, packed with 2.6 μm average diameter particles. A KrudKatcher ultra HPLC in-Line filter 0.5 μm was also used to protect column. Two microliters of sample were injected. The mobile phases were methanol:acetonitrile 80:20 (phase A) and ultrapure water 7 mM in formic acid (phase B), at a flow rate of 0.4 mL/min and were entirely transferred into the mass spectrometer source. The following gradient elution scheme was used: phase A was increased from the initial 0% to 15% in 0.2 min, then increased up to 35% in 3.2 min and after that was increased up to 100% in 2.6 min; 100% was maintained for another 2 min and then switched back to the initial 0% in 2 min. The complete separation of all substances occurs in 6 min. All the analytes were detected in positive ionization with a capillary voltage of 5500 V, nebulizer gas (air) at 40 psi, while the turbo gas (nitrogen) was at 90 psi and curtain gas was at 30 psi; the source temperature was 400 °C. For each analyte two multiple reaction monitoring (MRM) transitions were selected. Peak areas for the selected ions were determined using PESciex package Multiview 1.5 and quantitation was performed by the internal standard method. The selected transitions, together with the main LC–MS/MS parameters, are reported in Table 1.

4. Results and discussion

4.1. Virtual screening results

The hexapeptide was the result of a previous screening process in which more than 3500 receptors of 5 or 6 aminoacids long were generated and tested vs cocaine using a docking approach. The entire process is detailed in previous works [10,50] and summarized in Fig. 1.

In summary, the binding sites of 4 biological receptors for cocaine were analyzed; the aminoacids directly involved in the interactions with cocaine were used as a backbone in a semi-combinatorial way in order to decrease, by orders of magnitude, the number of combinations to be screened, and lowering significantly the molecular complexity in terms of sequence length and three-dimensional structure.

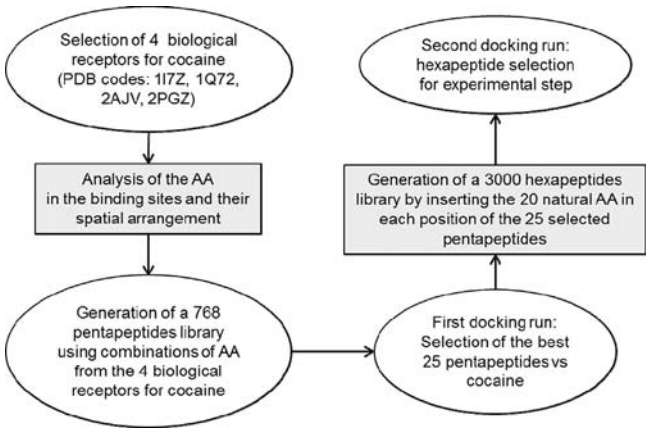

Fig. 1. Flowchart describing the process followed to obtain the hexapeptide used in this work. The method was described in detail in reference 50. AA=aminoacids.

The peptides were built taking into account only the primary structure of the biological receptors, reducing the size of the peptide to the minimum, in order to control the possible shape of the peptide secondary structure during the computational simulation. Due to the small size of cocaine and the spatial disposition of the aminoacids, it was possible to establish that the minimum length of the receptor peptides could be 5 aminoacids long. These peptides did not resemble any of the binding sites in the biological counterparts since the later were complex macromolecules, and the binding sites involved multiple chains. However, peptides contained the most representative aminoacids that interact with cocaine. Following this knowledge-based approach, in the first stage of the screening process, a library of 768 pentapeptides was built. After a first docking run versus the five drugs, a subset of 25 selected pentapeptides was used as seed for a second docking step in which the sequences were lengthen by the addition of one aminoacid, resulting in a 3000 hexapeptides library. The entire process was carried out considering both ligand and receptors flexibility.

The hexapeptide QHWWDW was designed at pH 4 and pH 7 with two or one NH bonds on imidazole ring in histidine residue. At pH 4.9 the hexapeptide had its isoelectric point and net charge at pH 7.0 was equal to −0.9. The hexapeptide showed poor water solubility with a strong aromatic portion and dual basic–acid properties. Fig. 2 shows the three-dimensional structures of hexapeptide at pH 4 and pH 7 along with the five drug ligands selected for this work.

In terms of modeling conditions, the hexapeptide conformational space was represented by ten conformers chosen as a good option to have fair speed-accuracy ratio. The overall tendency remained unchanged when using more conformers. A similar conformational trend behavior was also observed using peptides for dioxins family in previous work [10], reinforcing the possibility to adequately represent conformational space with 10 conformers.

To take into account the five drug ligands flexibility, a maximum of 200 conformers was allowed in OMEGA run, resulting in 34 conformers for cocaine, 14 and 21 conformers respectively for MDMA and MDEA, 2 conformers for phencyclidine, and no conformer for morphine due to its planar structure.

The FRED docking run was carried out comparing results obtained by the new version of FRED with the old one. In FRED old version, three computational parameters were evaluated:

exhaustive scoring (3 methods), optimization scoring function (8 methods) and standard scoring function (6 methods). In Tables 2 and 3 the resulting scores, expressed as binding percentage, of the hexapeptide at pH 4 and pH 7 vs. the 5 drugs ligands using docking methods combinations were reported. Except for default parameters, only computational method combinations that gave cocaine docking score better than other drugs were described. The data analysis was oriented to evaluate the hexapeptide specific response when docking cocaine in comparison with the other four drugs. It should be noted that in more than three other works, FRED default parameters were found to have the better convergence with experimental results [10,32,33].

In FRED default parameters, the hexapeptide designed at pH 4 showed highest affinity for ecstasy (both MDMA and MDEA) instead of cocaine. On the other hand, at pH 7 the hexapeptide showed a better behavior in binding cocaine in relation to other drugs, with a difference of almost 20% compared to the two ecstasies and a strong distinction, with a binding decrease of 50%, for both phencyclidine and morphine.

Fig. 3 represents the electrostatic molecular surface of the hexapeptide in complex with each drug. All drugs were docked in almost the same saddle shaded peptide region highlighting the role of histidine (in the left of peptide structure). In all cases analyzed, the third tryptophan residual group was oriented out of the interacting molecular surface.

The information obtained from three-dimensional shape and binding performances in different pH chemical environments was very useful to improve the SPE retention technique especially in attaching the peptide to the resin via carboxyl-terminus without losing the interaction properties.

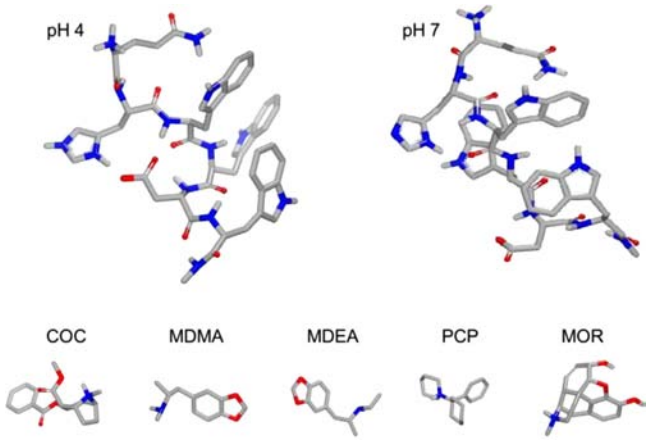

Fig. 2. Three-dimensional structures of hexapeptide QHWWDW at pH 4 and pH 7 along with the five drug ligands selected for this work. Peptide net charge at pH 7 = −0.9; Peptide isoelectric point = pH 4.94.

5. Experimental results

The cross reactivity experiments were carried out to test the affinity of the hexapeptide vs cocaine and other common drugs. Cocaine, MDMA, MDEA, phencyclidine and morphine were loaded at the concentration of 80 nM on the hexapeptide cartridge, under the same conditions optimized for cocaine in a previous work [19]. To evaluate nonspecific interactions between drugs and the stationary phase, cartridges were packed also with non-functionalized resin. The two SPE cartridges, with or without hexapeptide, were tested at pH 4 and pH 7 in order to find the best conditions for analyte retention and to evaluate as much as possible the environment calculated in virtual screening. The two tested loading conditions were acetate buffer at pH 4 and Tris–HCl buffer at pH 7. Different chemical environments were tested because hexapeptide was constituted by amino acids that possess protonation groups (histidine and aspartic acid), or hydrophobic groups such as the aromatic rings present on tryptophan and histidine. It should be highlighted that before their application, the cartridges were swelled and dried several times because it was

Table 2
Simulated scores, expressed as binding percentage, of hexapeptide QHWWDW at pH 4 (imidazole ring with one NH bond) in complex with COC, MDMA, MDEA, PCP and MOR. Results from FRED 3.0.0 and FRED 2.2.5 with different combination of docking methods were reported.

Method			COC %	MDMA %	MDEA %	PCP %	MOR %
Exhaustive scoring function	Optimization scoring function	Standard scoring function					
FRED 3.0.0 (default parameters)			89	97	100	80	71
FRED 2.2.5 (default parameters)			82	100	95	82	49
Sh	Nn	Sh	100	75	81	78	89
Sh	Nn	Ch2	100	66	73	71	78
Sh	Ch2	Sh	100	74	78	79	89
Sh	Ch2	Ch2	100	79	75	74	89
Sh	Ch3	Ch2	100	75	80	76	88
Sh	Ss	Sh	100	77	79	80	90
Sh	Ss	Ch2	100	78	82	80	87
Ch2	Nn	Ch2	100	79	78	72	82
Ch2	Sh	Ch2	100	71	75	72	86
Ch2	PLP	Sh	100	75	82	79	88
Ch2	PLP	Ch2	100	80	79	77	84
Ch2	Ch2	Ch2	100	80	84	74	86
Ch2	Ch3	Sh	100	74	81	81	89
Ch2	Ch3	Ch2	100	75	81	78	87
Ch2	Ss	Ch2	100	80	79	80	84
Ch3	Nn	Ch2	100	80	81	80	89
Ch3	PLP	Sh	100	77	77	81	89
Ch3	PLP	Ch2	100	79	75	79	83
Ch3	Ch2	Ch2	100	75	75	75	87
Ch3	Ch3	Ch2	100	73	75	78	89
Ch3	Ss	Sh	100	75	78	81	90
Ch3	Ss	Ch2	100	79	76	80	84

Table 3
Simulated scores, expressed as binding percentage, of hexapeptide QHWWDW at pH 7 (imidazole ring with one NH bond) in complex with COC, MDMA, MDEA, PCP and MOR. Results from FRED 3.0.0 and FRED 2.2.5 with different combination of docking methods were reported.

Method			COC %	MDMA %	MDEA %	PCP %	MOR %
Exhaustive scoring function	**Optimization scoring function**	**Standard scoring function**					
FRED 3.0.0 (default parameters)			100	83	79	55	60
FRED 2.2.5 (default parameters)			100	91	94	57	50
Sh	Nn	Sh	100	72	75	73	81
Sh	Nn	Ch2	100	81	77	69	84
Sh	PLP	Sh	100	70	77	77	87
Sh	Ch3	Ch2	100	88	85	77	90
Sh	Ocs	Sh	100	71	78	79	87
Sh	Ocs	Ocs	100	89	82	86	89
Sh	Ss	Sh	100	70	80	77	90
Ch2	Nn	Ch2	100	79	85	72	85
Ch2	Sh	Ch2	100	70	81	75	86
Ch2	Sh	Ocs	100	79	84	76	85
Ch2	Ch2	Sh	100	72	77	81	90
Ch2	Ch2	Ch2	100	82	85	73	89
Ch2	Ch3	Sh	100	71	78	81	87
Ch2	Ch3	Ch2	100	80	86	70	83
Ch2	Cs	Ch2	100	83	85	75	89
Ch3	Nn	Sh	100	70	78	79	90
Ch3	Nn	Ch2	100	71	74	71	85
Ch3	Sh	Ch2	100	75	76	70	86
Ch3	PLP	Ch2	100	78	84	73	84
Ch3	PLP	Ocs	100	83	89	80	87
Ch3	Ch3	Ch2	100	85	69	74	85
Ch3	Ocs	Ch2	100	79	80	74	85
Ch3	Ss	Ch2	100	83	68	77	89

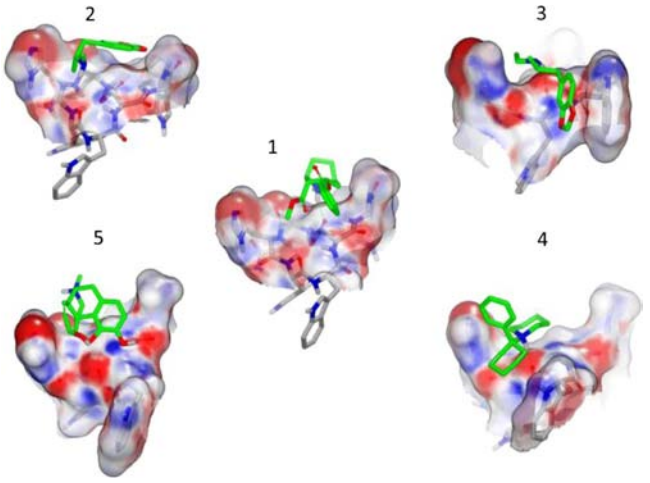

Fig. 3. Molecular surfaces of the hexapeptide QHWWDW in complex with drug ligands. 1-Hexapeptide-COC; 2-Hexapeptide-MDMA; 3-Hexapeptide-MDEA; 4-Hexapeptide-PCP; 5-Hexapeptide-MOR.

noted an improvement when cartridges were submitted to different cycles of conditioning and washing.

In the extraction procedure, the fourth step represents the bound analyte therefore this value was used to determinate the cartridges retention. Table 4 shows the cartridges response at pH 4 and 7 reporting the cocaine and other drugs retention average with their relative standard deviation. The experiments were carried out in triplicates using three different cartridges for each peptide and resins obtaining a reproducibility with a CV < 15%.

In all pH conditions and for all drugs, it can be noticed that functionalized hexapeptide resin exhibited a higher recovery when compared to the non-functionalized resin. Using acetate buffer at pH 4, the hexapeptide sorbent material showed a recovery of 81% for cocaine with a considerable difference from

Table 4
SPE cartridges recovery test at pH 4 and pH 7 for peptide and blank resin using 80 nM cocaine concentration. The coefficient of variation was in all cases no more than 15%.

Drugs	pH 4		pH 7	
	Peptide %	Blank %	Peptide %	Blank %
COC	81 ± 6	44 ± 6	97 ± 6	41 ± 4
MDMA	78 ± 6	36 ± 5	67 ± 11	38 ± 5
MDEA	76 ± 11	34 ± 4	71 ± 10	33 ± 4
PCP	55 ± 8	46 ± 5	60 ± 5	45 ± 7
MOR	50 ± 7	42 ± 6	45 ± 3	40 ± 5

recovery obtained using phencyclidine (55%) and morphine (50%) but not from recovery observed for both ecstasies (78% and 76%). The hexapeptide had a significant retention of cocaine only using Tris–HCl buffer at pH 7. According to the experimental data obtained at pH 7, the functionalized hexapeptide resin showed 97% of bounded cocaine more than 25% of recovery compared to ecstasies, 37% to phencyclidine and 52% to morphine. These results were in agreement with simulated data, since at pH 7 the best results were obtained using default parameters of both FRED versions.

The ability of functionalized hexapeptide resin to discriminate between cocaine and other drugs was demonstrated comparing the same extraction protocol at pH 7 using a commercial cartridge with a reversed phase functionalized polymeric sorbent with retention of neutral, acidic, or basic compounds. In Fig. 4 the comparative results in recovering the five drugs, between polymeric and hexapeptide stationary phases was reported. Also in this case, the average recoveries were calculated using three different cartridges and reported in percentage. The comparative analysis noticed that polymeric stationary phase exhibited similar recovery values for all the drugs tested, while a strong specificity for cocaine was achieved when using the hexapeptide.

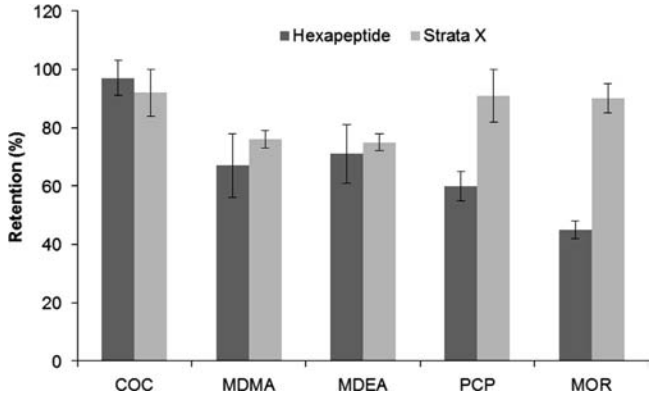

Fig. 4. Percentage retention of hexapeptide sorbent versus commercial polymeric sorbent (Strata X) bonding the five drugs evaluated in this work.

After checking the selective behavior of functionalized hexapeptide resin, the binding constant of the complex hexapeptide–cocaine was calculated by loading cocaine solutions at different concentrations ranging from 0.3×10^{-7} to 3.0×10^{-5} M. After detracting the unspecific bound contribution given by blank cartridge retention, the bound cocaine was determined by subtracting the free analyte from the total loaded. Considering 1:1 complexation stoichiometry, the ratio between bound and free cocaine versus the bound one was plotted and the binding constant was calculated by fitting a linear regression through these data [51]. The results of this procedure indicated a strong interaction between cocaine and hexapeptide with a binding constant of $2.9\times10^{6}\,M^{-1}$. Also in other works, the binding constants of short selective peptides showed this order of magnitude [52,53].

6. Conclusions

This approach confirmed the feasibility of bio-inspired peptide sorbent material which was selective for cocaine, compared with other similar drugs. Our procedure allowed to check the binding capacity of the peptide during the virtual screening process and to have a probable complex shape, avoiding large screening work.

The virtual binding scores obtained with the peptide at pH 7 were in good agreement with the experimental behavior. The preliminary studies on the pH optimization for SPE procedure were promising for the development of a new pre-concentration system with good recoveries. The bio-inspired sorbent material allowed to concentrate the samples and so its application could be useful in increasing analytical performances. Furthermore our approach to prepare bio-inspired recognition systems could be considered as a general method to obtain tailor-made reagents with antibody-like binding properties.

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