Computational aided acetaminophen – phthalic acid molecularly imprinted polymer design for analytical determination of known and new developed recreational drugs

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Abstract:

In recent times, abuse drug consumption rates have been increasing. In addition, authorities have detected a trend in the development of new substances expressly created to avoid legislation. These novel psychoactive substances (NPS) are non-registered formulations, closely chemically related to outlawed ones to maintain the same psychotropic effects while circumventing legal restrictions.

This issue arises enormous social, sanitary, and road safety problems since there is no way to detect nor quantify these non-registered substances.

The aim of this work is the development of a high selective material able to pre-concentrate and detect NPS. On that account, molecularly imprinted polymers (MIPs) designed with an imprinted cavity that matches the cathinones structural shape were proposed to detect both conventional and new cathinone derived recreational drugs.

The increasing number of illicit drug modifications that is being reported requires developing a re-ceptor valid for not only known molecules but also for incoming ones; thus, a virtual procedure must be carried out to take a step forward towards future modifications. Accordingly, a computational MIP design is proposed as the most appropriated method to effectively design this receptor.

By means of molecular dynamics and molecular docking, several combinations are studied regarding their pre-polymerization complex stability but also their rebinding capacity against the proposed analytes.

Hence, a phthalic acid – acetaminophen MIP is selected as the most well-suited receptor, valid for current and forthcoming cathinone recreational drugs.

Keywords:

Computational aided design (CAD); Dynamic simulation; Molecularly imprinted polymer (MIP); Cathinone; Bath salts

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Introduction

Nowadays, recreational drugs consumption is closely related to leisure, especially among young people. Consumption rates have been increasing since 2011, when the usage of drugs dropped dramatically, principally due to the economic crisis situation [1].

One of the most consumed drugs is khat, a native bush to Asia and Africa, and its derivatives, known as cathinones. Consumption of this kind of drugs has become popular online. Sellers offer them like bath salts which are easily bought in the black market using new currencies such as Bitcoin [1].

Development of new formulations is, as well as increasing consumption rates, one of the most disturbing issues of social, sanitary, and driving policies. These substances, which are not under international legislation control, are produced by means of small structural changes in the starting molecule in order to simulate the nervous central system effects of known drugs but bypassing the law [2].

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To prevent this problem, new analytical tools are needed, which allow the determination of these substances in concentrations at a trace level. The use of an analytical methodology based in molecularly imprinted polymer materials design is proposed. Based on the lock and key approach described by Fischer in 1894, a rigid matrix with cavities complementary in shape to the imprinting molecule was designed [3].

MIPs are synthesized from multicomponent mixtures of tem-plate (imprinting molecule), functional monomer, crosslinker and porogen; so, its optimization by traditional methods involves a considerable experimental work. Hence, to reduce experimental work and to anticipate the receptor to incoming needs to any new drug modifications, a computational aided MIP design is performed [4e7].

Cathinones are khat derivatives, but can also be synthetically produced; its modifications lead to mephedrone and pyrovalerone, which are also transformed in 3,4-methylenedioxymethanphetamine (MDMA) and methylenedioxypyrovalerone (MDPV) [8].

As is shown in Fig.1, MDMA and MDPV abuse drugs were modified to obtain new substances as JWH-007 ((2-Methyl-1-pentyl-1H-indol-3-yl)(1-naphthyl)methanone), JWH-018 (1-Naphthyl(1-pentyl-1H-indol-3-yl)methanone), JWH-047 ((1-Butyl-2-methyl-1H-indol-3-yl)(7-methyl-1-naphthyl)methanone), JWH-081 (4-Methoxy-1-naphthyl)(1-pentyl-1H-indol-3-yl)methanone) or XLR-11 ([1-(5-Fluoropentyl)-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)meth-anone), which, as of today, have been detected and outlawed [9,10].

In addition to the health risks the consumption of recreational drugs involves, there is also a related road safety problem. Increment of reaction times or hallucinations is responsible of numerous accidents. To overcome these problems, several programs like DRE, ROSITA or DRUID have been developed since 1970 aiming at improving drug detection methods [11e13].

Analytical determination of illicit drugs requires a specific procedure for each substance, normally carried out by liquid or gas chromatography - mass spectrometry determination (LC-MS, GC-MS) or a combination of both methods. This technology is quite time and cost-consuming since it requires an extensive sample preparation before each analysis and a high equipment investment [14e16].

Additionally, one of the most important determination-related problems is the fact that analysis must be extremely accurate, with high sensibility and specificity. Thus, high selective materials as MIPs, specifically designed for given analytes offer a good alternative as solid phase extraction materials (molecularly imprinted solid phase extraction, MISPE) or even as part of portable equipment able to measure analyte concentrations in situ [17e22].

MIPs require low sample volume to perform an effective anal-ysis, so this kind of material can overcome the arduous sample pre-treatment but also the low sample volume collection that is usually made during the roadside drug tests [23]. Furthermore, MIPs are much more economically feasible than the aforementioned traditional procedures. Thus, a high specific, low sample volume-requiring and low-cost device can be developed by using molecu-lar imprinting techniques [24,25].

Experimental

Reagent selection

As explained earlier on, cathinone and its derivatives, synthe-sized to avoid the legislation, are nowadays the most commonly consumed drugs [1,26]. Nevertheless, the current assay procedures for the determination of drug consumption involves a specific test for each substance, so new developed drugs cannot be easily detected. Due to this legal loophole, MIPs were proposed as alter-native materials to detect not a specific drug, but a structurally and electrostatically related family of drugs.

To obtain accurate results, detection and quantification limits must be as small as possible. Consequently, molecularly imprin-ted polymers were designed with mimic template molecules, which resemble molecules to cathinone, mephedrone and 3,4-methylendioxymethanphetamine (MDMA), and are employed to deliberately avoid false positive results due to the non-complete removal of the molecule employed during the imprinting step. Presuming the possibility of being used as on road drug detection devices which would be in contact with oral fluids or blood, mimic templates catechin, quercetin, nicotine, acetaminophen and ethoxyphenylacetamide were assayed with non-toxic func-tional monomers like itaconic acid, hydroxyethylmethacrylate (HEMA) and phthalic acid [27].

Equipment and programs

To develop polymerization computational simulations, Linux - based Ubuntu 15.04 64-bit OS was installed in an Asus PC with an Intel core i7-4790 CPU system of 3.60 GHz x 8 processors and 32 RAM GB.

Required programs were Openbabel [28], Acpype [29], Gromacs 5.0 [30,31], Chimera UCSF 1.10.2 [32], AutoDock Tools 4.2 [33], AutoDock Vina 2.0 [34], Pymol 1.7.2 (The PyMOL Molecular Graphics System, Version 1.7 Schr€odinger, LLC), Python 2.7.6 (Py-thon Software Foundation), MarvinSketch (ChemAxon) and HBo-nanza [35].

Molecular modelling

For pre-polymerization experiments, templates, mimic tem-plates, functional monomers and porogens were downloaded from Chemspider (www.chemspider.com)(Fig. 3). New proposed drug modifications A – F, derived from JWH-i, were designed with MarvinSketch (www.chemaxon.com)(Fig. 2). All molecules were processed following an energy minimization procedure using Openbabel with the General Amber Force Field (GAFF) [36] and following a steepest descent procedure with a maximum of 200,000 iterations.

Topology and parameter files were created using Acpype in a General Amber Force Field [36,37]. After the files were prepared, a 3 nm virtual box containing one template molecule was filled to maximum capacity with functional monomer molecules, using Gromacs 5.0 Molecular Dynamics Software Package [30,31].

To simulate the polymerization process, four minimization and two equilibration steps were performed [4]. The minimization process package is composed by two convergent gradient and two steepest descent protocols. It was employed to perform the rearrangement of the molecules to achieve the minimum energy of the set which is carried out during the real pre – polymerization mixture preparation. When the minimum energy was attained, a double equilibration step of 1,2 ns was performed to simulate, first, a temperature increment from 2 K to 333 K at atmospheric pressure and, second, a high constant temperature at 333 K at atmospheric pressure, which simulates temperature shift in real polymerization processes.

Then, hydrogen bonds were characterized using HBonanza Python Script so that polymer complex of template and monomers, formed during the simulation, could be isolated from the virtual box [35].

Chimera UCSF 1.10.2., an interactive program for visualization and inspection of molecular structures, was employed to measure the hydrogen bond distances that were previously isolated with HBonanza.

Affinity related to the hydrogen bonds between template and monomers was calculated with AutoDock Tools 4.2. To reproduce the experimental conditions of MIP synthesis, an organic environment was simulated for pre-polymerization studies.

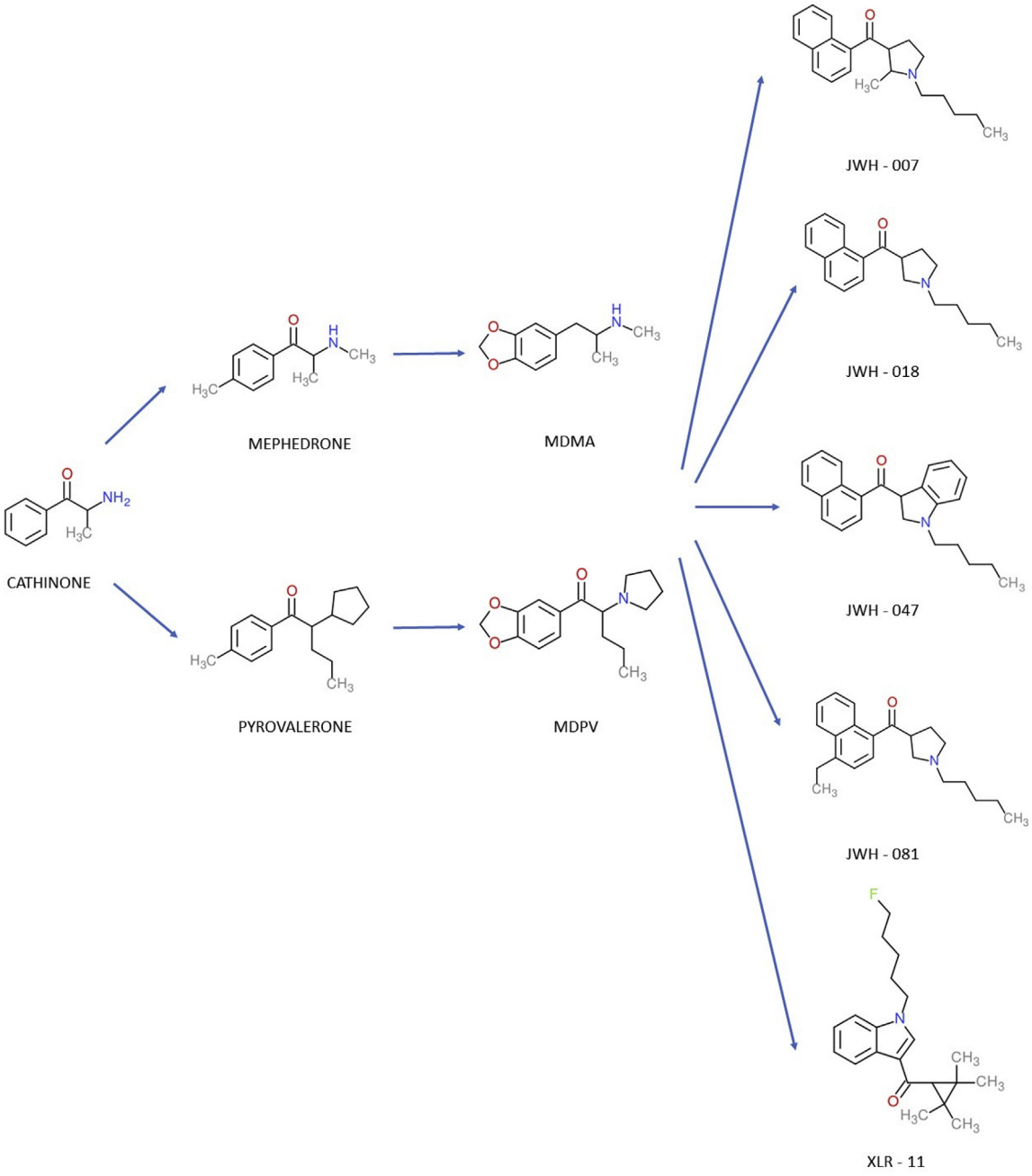

Fig. 1. Modification process of cathinone to avoid legislation.

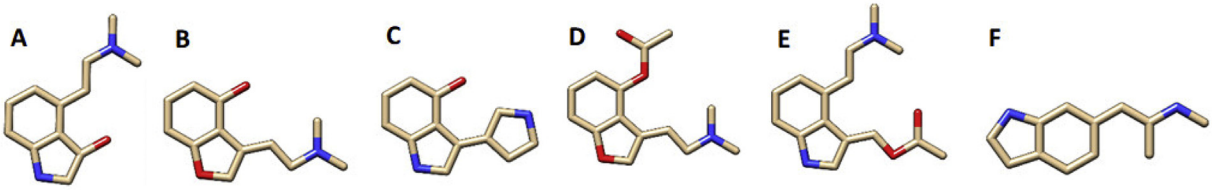

Fig. 2. Proposed modifications A to F (Red = Oxygen, Blue = Nitrogen). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The scoring function of this program is based in an Amber Force Field, so it was employed to measure the affinities of the pre-polymerization complexes that were obtained from the molecular dynamic simu-lations performed with Gromacs, carried out also in an Amber Force Field [37]. On that account, to obtain reliable quantitative binding energy values, same conditions were maintained during both mo-lecular dynamic simulation and molecular docking studies.

AutoDock Vina 2.0, based on a machine learning approach, was employed for post-polymerization studies. MIPs are expected to be used for on road devices, taking saliva samples for drug analysis. Correspondingly, the scoring function of the post-polymerization studies was adjusted to reproduce this aqueous environment where the rebinding process would take place [38].

Molecularly imprinted polymer design

To avoid false positive results during the experimental drug analysis, MIP receptors were designed with molecules similar in shape called mimic templates. In this study, catechin, quercetin, nicotine, acetaminophen and ethoxyphenylacetamide were employed to create the cavity in the rigid matrix. These mimic templates were selected due to their functional group similarities (acetaminophen and ethoxyphenylacetamide), structure and size similarity (catechin and quercetin) or both (nicotine). Hydroxyethylmetacrylate, itaconic acid and phthalic acid were used as functional monomers due to their biocompatible behaviour (Fig. 3).

These compounds were tested with the molecular modelling procedure explained in section 3.3., analysing hydrogen bonds and its related energy for each proposed receptor.

To correctly optimize the MIP receptor design, the porogen effect must be taken into consideration. High hydrogen bond energies of template – monomer complex are required, but the solvent effect must also be balanced. Hence, energy between mimic template and porogen should be weaker than mimic template and functional monomer, in order to avoid interferences in the imprinting process [39,40]. This energy is also analysed with AutoDock Tools 4.2.

Binding evaluation

Once the bond energy analysis of the mimic template – monomers complexes was performed, the mimic template was removed and a binding evaluation of the remaining receptors was carried out.

These empty rigid MIP receptors were tested against emergent and newly consumed drugs, and against new possible modifications proposed proposed by modifying the already existent drugs (Figs. 2 and 3). With this purpose, MIPs formulations were tested to obtain the best combination to adsorb a family of molecules similar in shape.

Results and discussion

As indicated in the introduction, the development of new cathinone-based drugs of abuse is ongoing by different criminal organizations. This means that when new analytical methodologies are developed for the determination of these compounds, they cannot be designed only for those compounds that are currently present on the illegal market, but also for those that, after a small modification, may give rise to new varieties. Molecular modelling is a perfect tool for this purpose, as it allows working with virtually designed molecules. Therefore, in this discussion of results we start by presenting the results obtained in the work developed from an approach based on computational chemistry. To finish with the results obtained in the experimental tests in the laboratory, which will allow us to evaluate the capacity of the imprinted polymers, to selectively absorb these new substances and thus concentrate them in a previous clean-up stage in the development of a new analytical method.

Analysis of mimic template – monomer ratio and bond energies determination

The design of these MIP drug receptors involves a first step of studying the pre – polymerization complex formed by the pro-posed mimic templates and monomers. Number and energy of the hydrogen bonds between one mimic template and several monomers is determined as previously explained in sections 3.3. and 3.4. (Table 1).

Results show that combinations of phthalic acid are expected to be the most well – suited to adsorb the studied recreational drugs since their structurally similar counterparts present higher number of hydrogen bonds and higher affinities for combinations with this monomer.

This assumption is tested according to the protocol explained in the binding evaluation section 3.5.

Rebinding analysis

As mentioned before, to define cavities similar in shape for drug rebinding, mimic templates catechin, acetaminophen, nicotine, ethoxyphenylacetamide and quercetin are proposed.

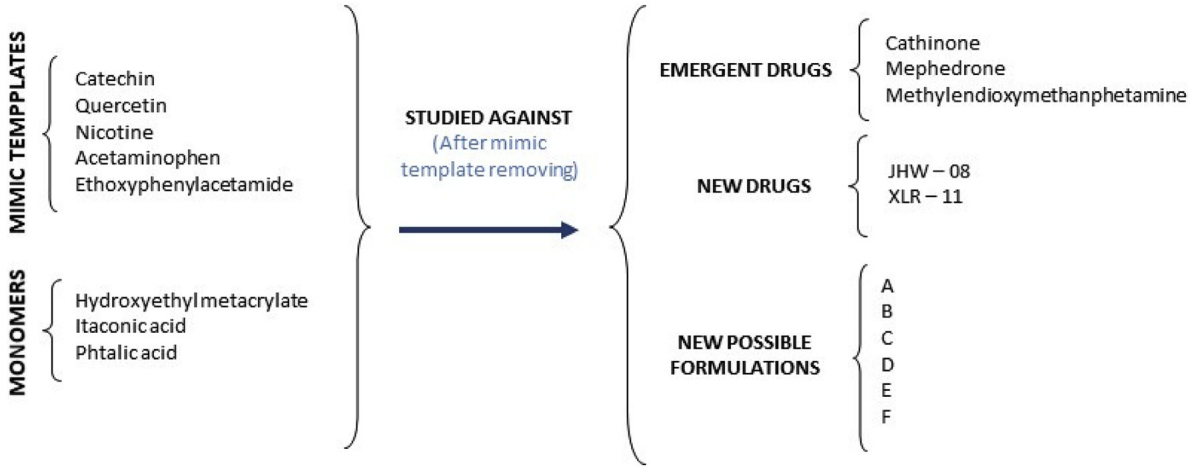

Fig. 3. Receptor design process scheme.

Table 1
Number of hydrogen bonds and affinities (Kcal/mol) present in mimic template – functional monomer complexes.

Mimic template	FUNCTIONAL MONOMER					
	HEMA		Itaconic acid		Phthalic acid	
	H Bond	E (kcal/mol)	H Bond	E (kcal/mol)	H Bond	E (kcal/mol)
Catechin	5	−13.8	7	−18.6	7	−23.5
Acetaminophen	3	−11.2	3	−11.2	5	−22.1
Nicotine	–	–	2	−10.2	1	−12.8
Ethoxyfenilacetamide	1	−6.0	1	−6.1	2	−9.0
Quercetin	3	−10.4	4	−18.5	5	−20.3

Hydrogen bonded formed complexes between these mimic templates and functional monomers were isolated. Then, mimic templates were removed and rebinding studies of proposed drugs (emergent, new, habitual and own-prosed) (Figs. 3, 4) are carried out in these empty rigid receptors.

Results are summarized in Table 2. Quantitative analyses of affinities between mimic templates and monomers show that formulations with phthalic acid are the most suitable for catechin, acetaminophen, nicotine and ethoxyphenylacetamide, but itaconic acid has higher affinity values for quercetin imprinting.

Catechin and acetaminophen in combination with phthalic acid imprinting receptors and quercetin in combination with itaconic acid imprinting receptor, present significant higher affinties than the nicotine and ethoxyphenylacetamide ones. Accordingly, these last two receptors were withdrawn from further studies. The small affinity differences between the catechin – phthalic acid, acet-aminophen – phthalic acid and the quercetin – itaconic acid combinations make it not possible to discern them clearly, so their correspondent empty receptors were studied against new pro-posed modifications of already existent drugs (Fig. 3).

Table 3 shows that obtained affinity energies for catechin – phthalic acid receptor are slightly higher, but values for the other two studied receptors are quite similar, so these results disregard choosing one of them as the best MIP combination.

By and large, the number of hydrogen bonds is a key parameter to choose between different MIP formulations. Therefore, pre – polymerization complexes with high number of hydrogen bonds between template and functional monomers are expected to show better performances during the rebinding study, as they offer more functional groups well-oriented to accommodate rebound molecules.

In this case, quercetin – itaconic acid MIP has 5 hydrogen bonds and 4 surrounding monomers (Fig. 5A), catechin – phthalic acid MIP has 7 hydrogen bonds (one for each monomer) (Fig. 6A) and acetaminophen – phthalic acid has 5 hydrogen bonds (one for each monomer) (Fig. 7A). These results indicate that catechin – phthalic acid MIP receptor is expected to show a better performance during the rebinding studies; to contrast this hypothesis, rebinding posi-tions were analysed with AutoDock Vina 2.0 (Figs. 5B, 6B and 7B).

Catechin imprinting receptor was expected to present better adsorptive characteristics than the others due to its higher number of functional monomers, but Fig. 6B shows that rebound molecules bind to this receptor not in the defined cavity but in the posterior part of it. Nevertheless, both acetaminophen – phthalic acid and quercetin – itaconic acid receptors perform adsorption processes into the right defined rebinding cavity (Figs. 5B and 7B, respectively).

Contrasting acetaminophen and quercetin cavities, the higher monomer number present in the acetaminophen receptor creates a more well-defined cavity, with higher number of hydrogen bonds surrounding rebound molecules (Fig. 7B). Despite of their closely affinity scores, the acetaminophen – phthalic acid MIP receptor formulation was expected to be the best candidate to perform the adsorption of the proposed drug family. Nevertheless, to make a correct decision between these two remaining receptors, a study against biocompatible porogens is required.

Porogen selection

Bond affinities between the pre – polymerization complex and the porogen must be balanced to promote the imprinting process during the experimental procedure. High affinity between template and functional monomer is required to create a stable pre-polymerization complex, but this purpose also implies a high af-finity requirement between template and porogen so that a good solubilization of the template molecule can be obtained.

Template – monomer affinity must be slightly higher than the template – porogen one to prevent the template from being bound preferentially to the porogen, causing low imprinting rates due to the non pre – polymerization complex formation.

In this study, an affinity analysis with AutoDock 4.2 was per-formed to correctly select the most appropriated porogen (Table 4). Acetone and ethanol were proposed as biocompatible solvents.

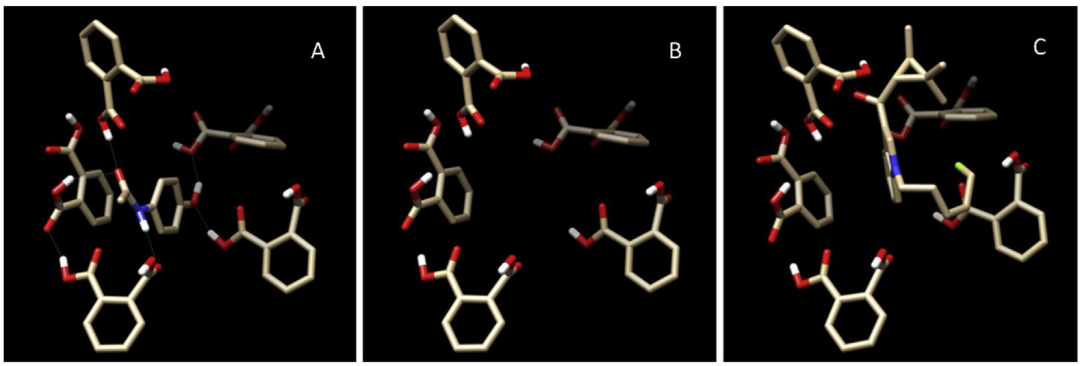

Fig. 4. A) Hydrogen bond determination in acetaminophen – phthalic acid complex. B) Acetaminophen – phthalic acid receptor after template removal. C) XLR-11 rebinding in conformed receptor.

Table 2
Receptor affinity against most consumed and recent drug variations (Kcal/mol).

		Functional monomer													
		Hema	Itac. ac.	Phth. ac.	Hema	Itac. ac.	Phth. ac.	Hema	Itac. ac.	Phth. ac.	Hema	Itac. ac.	Phth. ac.	Hema	Itac. ac.	Phth. ac.
	Mimic template	Catechin			Acetaminophen			Nicotine			Ethoxyphenylacetamide			Quercetin		
STUDIED DRUGS	**Cathinone**	−10.9	−13.8	−18.6	−9.5	−10.0	−18.4	–	−8.8	−11.8	−8.5	−7.7	−11.9	−9.4	−18.1	−15.6
	Mephedrone	−8.8	−11.8	−14.7	−7.5	−8.4	−15.7	–	−7.1	−9.9	−6.9	−6.5	−10.0	−7.9	−14.8	−13.1
	MDMA	−9.4	−12.2	−16.5	−8.3	−8.5	−16.0	–	−7.6	−8.9	−6.8	−6.5	−9.5	−7.7	−14.7	−12.8
	JWH-018	−8.5	−10.7	−15.7	−6.4	−7.1	−15.5	–	−7.7	−7.4	−5.2	−4.9	−8.4	−7.6	−13.2	−13.1
	XLR-11	−6.2	−7.3	−11.4	−5.1	−5.2	−10.0	–	−5.0	−5.4	−4.2	−3.8	−5.6	−5.2	−11.6	−13.1

Table 3
Receptor affinity against own proposed modification of cathinone − based drugs (Kcal/mol).

–	Itaconic acid	Phthalic acid	
	Quercetin	Catechin	Acetaminophen
A	−17.9	−19.3	−16.8
B	−17.0	−17.6	−17.5
C	−28.4	−29.9	−29.8
D	−14.2	−14.5	−14.3
E	−12.8	−13.4	−12.8
F	−18.3	−19.0	−18.3

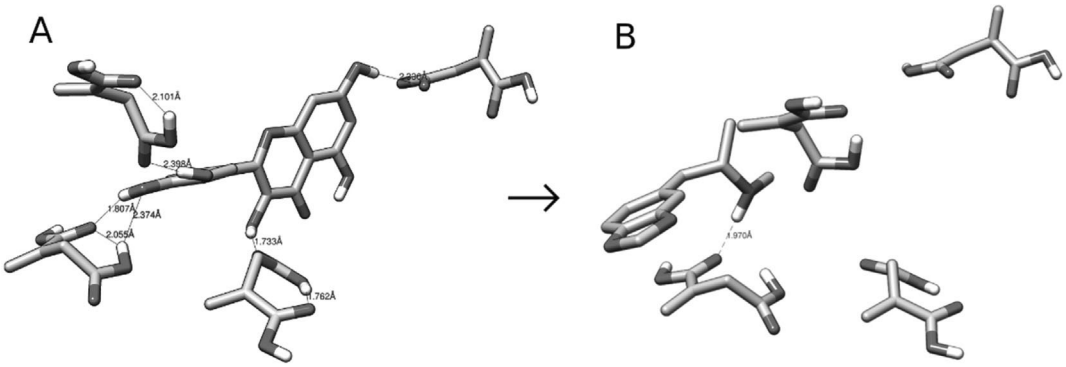

Fig. 5. A) Quercetin − itaconic acid pre − polymerization complex. B) Rebinding MDMA in quercetin − itaconic acid receptor.

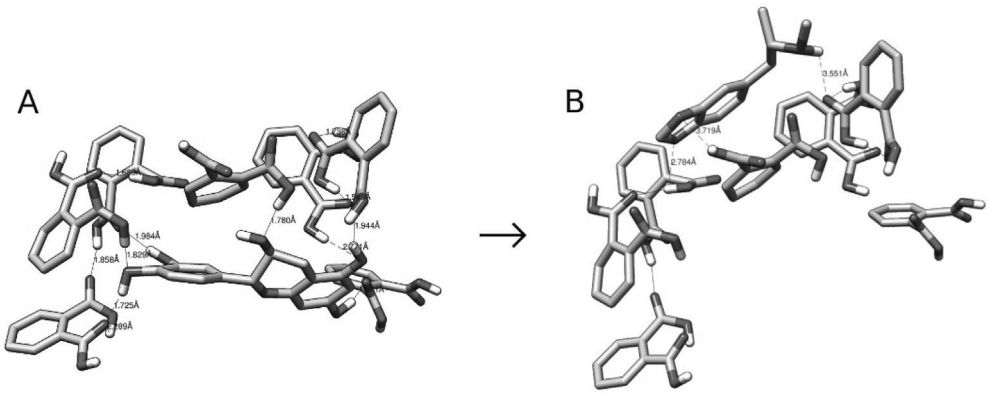

Fig. 6. A) Catechin − phthalic acid pre − polymerization complex. B) Rebinding MDMA in catechin − phthalic acid receptor.

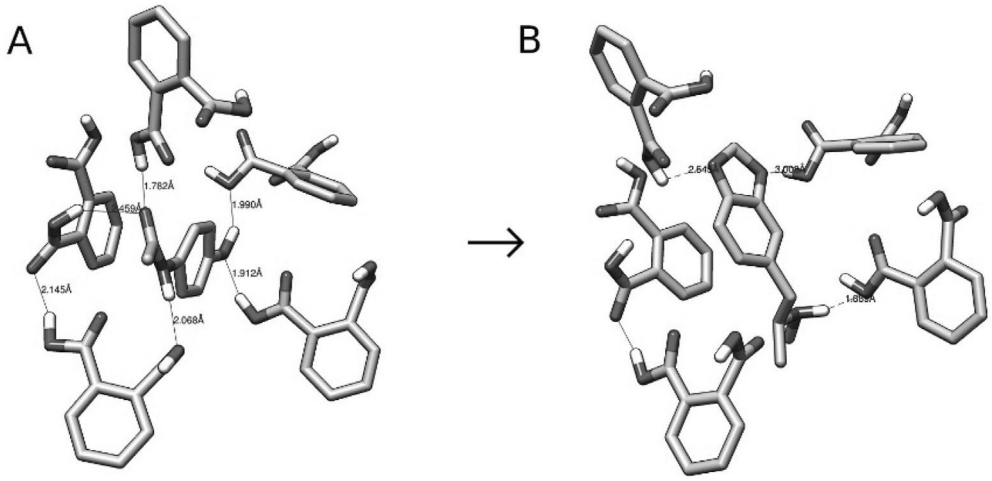

Fig. 7. A) Acetaminophen – phthalic acid pre – polymerization complex. B) Rebinding MDMA in acetaminophen – phthalic acid receptor.

Table 4
Affinities present in the acetaminophen – phthalic acid and the quercetin – itaconic acid receptors (Kcal/mol).

	Acetaminophen receptor	Quercetin receptor
Template - Monomer	−49.9	−40.5
Template - Acetone	−15.9	−16.3
Template - Ethanol	−26.4	−26.7
Monomer - Acetone	−14.1	−19.4
Monomer - Ethanol	−24.6	−26.3

The obtained affinity energies are less favourable for ethanol. This implies that a good pre – polymerization complex between template and monomer is not expected for this porogen, since the favourable affinities between template – ethanol and monomer – ethanol would cause low imprinting efficiency during the poly-merization process.

Affinity between acetaminophen and phthalic acid is higher than between quercetin and itaconic acid. Thus, a better imprinting process is expected for this pre – polymerization complex of acetaminophen – phthalic acid in acetone.

These results, in addition to the rebinding analysis (Fig. 7B), show that acetaminophen – phthalic acid – acetone formulation will conform the most appropriated MIP receptor to adsorb the proposed family of drug molecules.

Conclusions

Computational methodologies are a promising tool for materials design. The time and cost consuming methodologies of assay and error could be greatly simplified by using this technology, since it makes it possible to test hundreds of different combinations before accessing the laboratory.

In this study, an acetaminophen – phthalic acid MIP synthesized in acetone was suggested as a biocompatible material able to adsorb standard and arising recreational drugs. Adapted to a sensor device or employed directly to collect oral fluid, this receptor will be helpful to reduce the extensive sample pre – treatment and analysis that are carried out nowadays during roadside drug tests.

Similar results are obtained both for usual drugs and proposed virtual modifications of them; then, the system is considered valid not only for current outlawed drugs but also for possible future variations to circumvent the law.

Computational methodologies have been proved as a useful tool to assay virtual molecules that cannot be analysed otherwise, helping authorities and researchers to take a step forward drug consumption trends.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmgm.2020.107627.

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