

Computational Screening of Beverage Components and Machine-Learning Classification of a Multi-Zone Colourimetric Indicator for Drink-Spiking Detection

Ranbir Chadha

Abstract

Drink spiking with sedative drugs is a common and under-reported form of drug-facilitated sexual assault, and the drugs involved are often colourless, odourless and hard to taste in a drink. Current detection relies either on lab instruments such as GC-MS or on commercial kits that are expensive, test for only one drug class, and often give unreliable results in cocktails, citrus drinks and other complex beverages. No cheap strip exists that can detect several spiking drugs by eye. This study used a computational approach to find a drink component that could serve as the recognition element of such a strip. Five spiking drugs (GHB, alprazolam, clonazepam, diazepam and zolpidem) were docked in AutoDock against five common beverage components (citric acid, cyanidin-3-glucoside, ethanol, ethyl acetate and gallic acid). SwissADME was then used to calculate hydrogen-bond donors and acceptors, topological polar surface area, consensus LogP and water solubility class for each molecule. Docking showed that cyanidin-3-glucoside bound most strongly to all five drugs, while GHB was the weakest-binding drug. The descriptor results matched this pattern: cyanidin-3-glucoside has many hydrogen-bonding sites and a large polar surface area, whereas GHB is small, with few functional groups and no aromatic ring. Because cyanidin-3-glucoside is an anthocyanin whose colour already shifts with pH, it is proposed as a candidate recognition element for a colourimetric drink-spiking strip. A separate method will probably be needed for GHB.

Introduction

The term drink spiking refers to the act of adding a drug or alcohol into someone's drink without their knowledge or permission. This act is often done to incapacitate the victim and is a common route to crimes such as theft or sexual assault [1]. Drink spiking is also badly under-reported. In the United Kingdom, 2.2% of adults said they had a drink spiked in the previous twelve months, yet around 90% of them did not contact the police (Gautam & Grela, 2024).

The substances chosen for this purpose are selected precisely because they are difficult to detect by sight, smell or taste, generally termed "date-rape drugs". The most prevalent among these are gamma-hydroxybutyric acid (GHB), which is colourless, odourless and tasteless; benzodiazepines such as flunitrazepam (Rohypnol), diazepam and midazolam, which dissolve invisibly into a beverage; and ketamine and scopolamine [2]. Although they work through different biological pathways, they converge on the same outcome: rapid depression

of the central nervous system, producing drowsiness, loss of motor control and anterograde amnesia, so that the victim is unable to resist the crime and frequently cannot recall it afterwards.

In previous studies, it has been identified by Gautam et al. (2014) that benzodiazepines in spiked drinks can be detected reliably by gas chromatography–mass spectrometry, although the method needs heavy laboratory equipment. Also, a problem they identified is that flunitrazepam and temazepam degrade substantially over a short period of time within the drink itself, indicating that the detection window is finite. It has been shown by Son et al. (2021) that a paper sensor made with gabazine-linked polydiacetylene turns from blue to red when GHB is present, even in coloured and alcoholic drinks. Ryu and Kim (2022) detected GHB with a BODIPY–Fe³⁺ indicator displacement assay, which changes colour and fluorescence and is less affected by the other ingredients in a drink. It has been reported by Suleman et al. (2024) that a paper-based electrochemical device carrying a ketamine-specific aptamer can detect ketamine in alcoholic and non-alcoholic drinks, though its signal has to be read with an instrument. Gautam and Grela (2024) also found that commercial drink-testing kits gave false positives in plain water and missed spiking in cocktails.

Taken together, these studies show detection moving forward one drug at a time. Each sensor targets a single drug or drug class, several need an instrument to read, and in each case the recognition chemistry was found by experiment rather than by screening candidates first. No low-cost, instrument-free indicator yet exists that can screen one drink for several classes of spiking drug and still respond to drugs it was not built for. This study approached that gap in two stages. Molecular docking and descriptor analysis were first used to screen common beverage components for a natural compound that binds spiking drugs and could serve as a colour-change reagent. A machine-learning model was then trained to read the combined response of a multi-zone colour indicator. It was hypothesised that a multi-zone indicator read by a trained classifier would separate spiked from unspiked drinks with high accuracy across different drink types, including drinks spiked with drugs left out of training.

Methodology

Data Collection

Data collection began with a literature review, which was used to decide which drugs and drinks the investigation would cover. For the drugs, the substances that turn up most often in drink-spiking cases were identified. These were GHB and a group of sedatives: the benzodiazepines alprazolam, clonazepam and diazepam, and zolpidem. Zolpidem is a Z-drug rather than a true benzodiazepine, but it is used in the same way. The beverages most commonly linked to spiking cases were chosen while selecting the drinks: water, soft drinks,

beer, wine, spirits, and mixed drinks. While formulating both lists, case reports were used in order to follow how spiking actually occurs in the real world, instead of random drink-drug pairings. Next, each drug and drink was broken down into its own specific chemical components. For drugs, the functional groups of each molecule were recorded, such as the diazepine ring and imine group of the benzodiazepines. For drinks, the main components of each beverage were listed in an organised fashion. Both sets were organised into tables, and the number of times each chemical feature appeared was tallied. This revealed the components most common to drinks, and the chemical features shared by most drugs. After this, molecular docking was used to study how the drug and drink components interact.

Online chemical databases, such as PubChem, were the main sources for data on substances. For each drug, the molecular formula, structure, and functional groups were noted, and the three-dimensional structure was downloaded as an SDF file. The SDF file describes the full shape of the molecule, as it stores the position of every atom and the bonds between all the groups present. Similarly, the beverage components were looked up on PubChem, and the docking-target structures were taken from the RCSB Protein Data Bank in the PDB file format.

Molecular Docking

Next, molecular docking was used to measure how strongly each drug binds to each drink component. The method predicts how two molecules fit together and estimates the strength of the fit. This docking was carried out with the Smina software on the Tamarind Bio platform. For every combination, the structure files were first uploaded, and each Smina task was set with the drug as the ligand (in SDF format) and the beverage compound as the target (in PDB format). A grid box was defined each time using the x, y, and z axes, and the dimensions. Apart from the grid box size, all other settings were kept the same for all runs. If they were changed, it would be impossible to compare the results. Smina works by generating several possible orientations of the drug and beverage component, scoring how well each one fits. The binding affinity is returned in kcal/mol. A more negative value means a stronger and more stable interaction. Every binding affinity value for each drug and drink component was recorded in order in an Excel spreadsheet. This allowed for the binding affinity values to be compared directly.

Molecular Descriptor Analysis

Alongside the docking carried out, SwissADME was used to calculate a set of helpful molecular descriptors for each drug and beverage component. This assisted in describing their chemical and physical properties more accurately. For each compound, its SMILES string was found by searching for the compound on PubChem. Ten strings were collected: 5 for the

drug components and 5 for the beverage components. Each string was entered into SwissADME, which reads the structures from the notation itself. Five values were recorded for every compound: the number of hydrogen-bond donors, the number of hydrogen-bond acceptors, the topological polar surface area (TPSA, in Å²), the consensus LogP, and the predicted water solubility class. Consensus LogP measures lipophilicity and is the average of several prediction methods.

Model Building

After performing molecular docking and molecular descriptor analysis, a supervised machine learning model was built to classify between spiked and non-spiked beverage samples. The model was trained on a multi-channel sensor response dataset which was divided into training and test sets in the ratio of 80:20. A validation dataset was also built. Features included 14 sensor response variables, beverage type, drug components and the concentrations. An XGBoost classifier was used to train the model. AUROC values and AUPRC values, along with F1 score, precision, and recall, were computed to check the efficiency of the model.

Results

Molecular Docking Analysis

Molecular docking is a computational method that predicts how a small molecule (the ligand) binds to a target molecule and estimates how strong that binding is. As mentioned above, the software works by positioning the ligand against the target in multiple different possible orientations and scores how each one fits. The strength of this fit is expressed as the binding affinity (kcal/mol). While a negative value correlates to a stronger, more stable binding (since more energy is released when the molecules come together), a positive value means the two do not bind favourably. The grid box was set to $20 \times 20 \times 20$ for larger molecules like cyanidin-3-glucoside and $10 \times 10 \times 10$ for small ones like ethanol.

Table 1 gives the binding affinity for each pair, in kcal/mol.

Table 1. Binding affinity (kcal/mol) of each drug component docked against each drink component.

Ligand (drug component)	Receptor (drink component)	Binding affinity (kcal/mol)
GHB	Citric Acid	-1.6
GHB	Ethanol	2.2
GHB	Cyanidin-3-Glucoside	-2.6
GHB	Ethyl Acetate	-1.0
GHB	Gallic Acid	-1.5
Diazepam	Citric Acid	-2.3

Ligand (drug component)	Receptor (drink component)	Binding affinity (kcal/mol)
Diazepam	Cyanidin-3-Glucoside	−4.2
Diazepam	Ethyl Acetate	−1.8
Diazepam	Gallic Acid	−2.7
Zolpidem	Citric Acid	−2.0
Zolpidem	Ethanol	−1.0
Zolpidem	Cyanidin-3-Glucoside	−4.3
Zolpidem	Ethyl Acetate	−1.5
Zolpidem	Gallic Acid	−2.4
Clonazepam	Citric Acid	−2.3
Clonazepam	Ethanol	−1.2
Clonazepam	Cyanidin-3-Glucoside	−4.3
Clonazepam	Ethyl Acetate	−1.6
Clonazepam	Gallic Acid	−2.5
Alprazolam	Citric Acid	−2.4
Alprazolam	Ethanol	−1.3
Alprazolam	Cyanidin-3-Glucoside	−4.2
Alprazolam	Ethyl Acetate	−1.8
Alprazolam	Gallic Acid	−2.9

The strongest interactions across all drugs were with cyanidin-3-glucoside, the anthocyanin pigment, which produced the most negative affinities in the set. For the sedatives, it was −4.3 kcal/mol with zolpidem, clonazepam and alprazolam, and −4.2 with diazepam. The four sedatives behaved very similarly to one another, each binding cyanidin-3-glucoside most strongly, gallic acid and citric acid moderately, and ethanol most weakly. GHB stood out as different: it bound weakly to all five components, with its strongest interaction (cyanidin-3-glucoside, −2.6 kcal/mol) still weaker than the other drugs' weakest, and its pairing with ethanol giving a positive value (+2.2 kcal/mol), meaning no favourable binding occurred. Overall, ethanol was the weakest-binding drink component throughout, while the larger, more structurally complex components, cyanidin-3-glucoside in particular, bound the drugs most strongly.

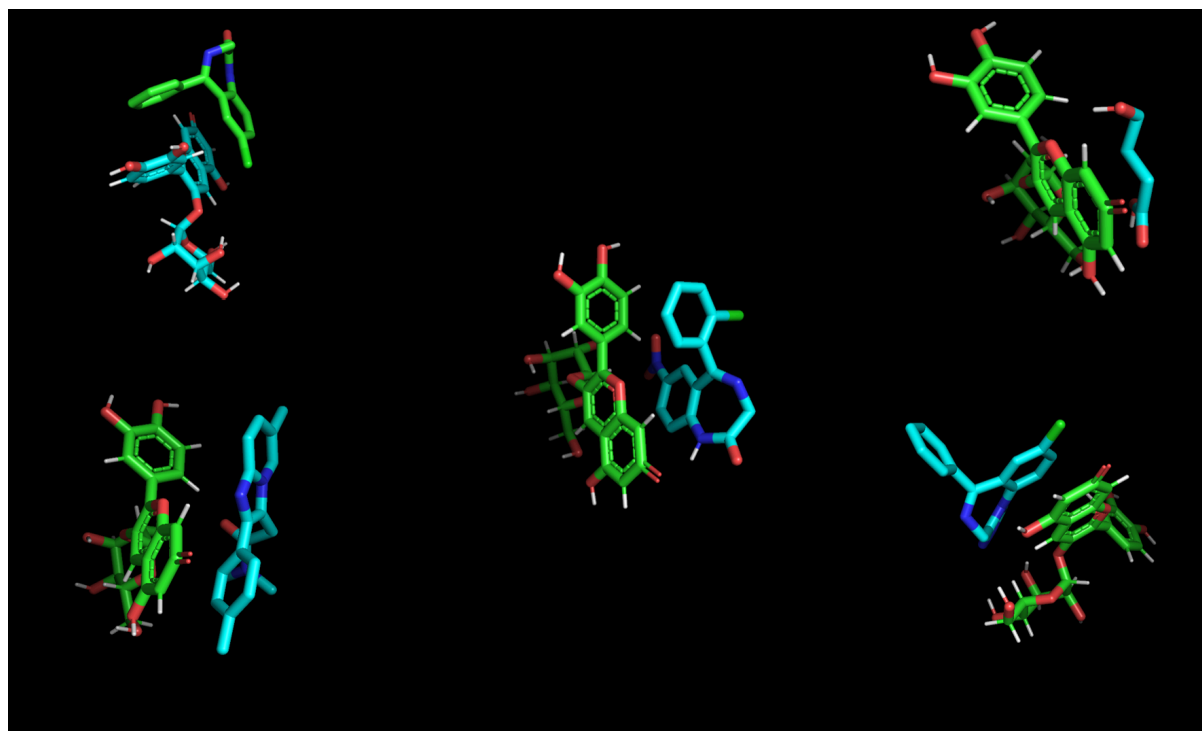


Figure 1. Molecular docking interactions between cyanidin-3-glucoside and selected psychoactive compounds. Three-dimensional molecular representations of the docking complexes formed between cyanidin-3-glucoside and five selected compounds: (A) diazepam, (B) GHB, (C) zolpidem, (D) clonazepam, and (E) alprazolam. Cyanidin-3-glucoside is shown in cyan, while the respective co-docked compounds are shown in green. Atoms are represented using standard element-based colouring, with oxygen in red, hydrogen in white, nitrogen in blue, and chlorine in green. The figure illustrates the predicted spatial association and molecular orientation of cyanidin-3-glucoside with each compound following molecular docking.

Molecular Descriptor Analysis

Five molecular descriptors were chosen to characterise each compound, each describing a different aspect of its chemical behaviour. The number of hydrogen-bond donors refers to the hydrogen atoms bonded to nitrogen or oxygen that can be donated to form a hydrogen bond, while the number of hydrogen-bond acceptors refers to the nitrogen and oxygen atoms with a lone pair available to accept one. The topological polar surface area (TPSA), measured in Å², is the total surface area of the molecule made up of polar atoms, mainly nitrogen and oxygen; it indicates how polar the molecule is and how well it can interact with water and with polar binding sites. The consensus LogP is a measure of lipophilicity, how readily the molecule dissolves in fat rather than water, where a higher value means the molecule is more lipophilic. Finally, the water solubility class is a predicted category, such as soluble, moderately soluble, or poorly soluble, describing how well the molecule dissolves in water.

This analysis was carried out to describe the physical and chemical properties of each drug and beverage component, and to help explain the docking results. The way two molecules

bind depends on properties such as their polarity and their ability to form hydrogen bonds, so these descriptors provide a chemical basis for understanding why some drug–component combinations bound more strongly than others. The data collected are shown in Table 2.

Table 2. Molecular descriptors calculated in SwissADME for each drink component and drug.

Molecule	H-bond donors	H-bond acceptors	TPSA (Å ²)	Consensus LogP	Water solubility class
Citric Acid	4	7	132.13	−1.51	Very Soluble
Cyanidin-3-Glucoside	8	11	193.44	−1.18	Very Soluble
Ethanol	1	1	20.23	−0.16	Highly Soluble
Ethyl Acetate	0	2	26.30	0.52	Very Soluble
Gallic Acid	4	5	97.99	0.21	Soluble
Alprazolam	0	3	43.07	2.84	Soluble
Clonazepam	1	4	87.28	1.98	Soluble
Diazepam	0	2	32.67	3.04	Soluble
GHB	2	3	57.53	−0.38	Very Soluble
Zolpidem	0	2	37.61	2.86	Moderately Soluble

Model Evaluation

The XGBoost classifier demonstrated strong discrimination between spiked and non-spiked beverage samples. It achieved an AUROC value of 0.964 and an AUPRC value of 0.976 on the validation set. On the test set, the AUROC value was 0.950, and the AUPRC value was 0.965. Using the validation-selected probability threshold, the model achieved an overall test accuracy of 88.2% and a validation accuracy of 88.3%. Out of the 559 spiked test samples, 488 were correctly identified, and 71 were incorrectly identified. For the 441 non-spiked samples, 394 were correctly classified, and 47 were misclassified. This corresponds to a precision of 91.2%, recall of 87.3% and F1 score of 89.2%.

Discussion

A computational workflow was used in this study to find a compound that is already found in drinks and could work as the recognition element of a colourimetric strip for drink-spiking drugs. Five drugs often linked to drug-facilitated sexual assault were chosen: GHB, alprazolam, clonazepam, diazepam and zolpidem. These were paired with five common drink components, picked so that each main chemical type in beverages was represented: citric acid, ethanol, ethyl acetate, gallic acid and the anthocyanin cyanidin-3-glucoside. Structures and SMILES strings were taken from PubChem. Docking was then run in AutoDock, since it

allows every drug–compound pair to be screened quickly and for free before any lab synthesis is attempted. Cyanidin-3-glucoside bound most strongly to all five drugs. This makes sense given its structure. The hydroxyl groups on its catechol ring and glucose unit can hydrogen-bond with the carbonyl, imine and triazole groups of the benzodiazepines and with the amide group of zolpidem, and its flat, conjugated ring system may stack against the drugs' aromatic rings. GHB bound the most weakly of the drugs, which was expected for a small aliphatic molecule with only two functional groups and no aromatic ring. To explain these results, SwissADME was used to calculate hydrogen-bond donors and acceptors, topological polar surface area, consensus LogP and water solubility class. Compounds with more hydrogen-bonding sites and a larger polar surface area tended to bind more strongly, which fits with the docking results.

Other groups have worked on colourimetric detection of spiking drugs using quite different methods. Ryu and Kim (2022) detected GHB with a BODIPY–Fe³⁺ indicator displacement assay, where GHB pushes the metal out of the complex and changes its colour and fluorescence. Suleman et al. (2024) built a paper-based electrochemical device that used an aptamer to bind ketamine, with the signal read by voltammetry. Both groups optimised one drug–receptor pair by experiment alone. The present study, by comparison, screened several possible receptors against several drugs on a computer before making anything, and it aims for a colour change visible to the eye rather than a reading that needs an instrument. Two other studies are closer to the approach used here. Son et al. (2021) used quantum-chemical simulation to study how gabazine binds GHB, then built a paper sensor that turns from blue to red and could detect GHB in coloured and alcoholic drinks. Wu et al. (2025) used docking to check how a screened aptamer bound the pesticide deltamethrin, finding a favourable binding energy of -7.35 kcal/mol, and then developed a cheap colourimetric test, readable by eye, based on gold nanoparticle aggregation. As in those two studies, modelling was used here to justify the choice of recognition element before building a sensor. The difference is that the candidate chosen here is a natural, food-safe pigment that could produce the colour change itself.

However, there are several limitations to the study as well. The docking was performed with Smina, which was developed primarily for protein–ligand systems. The affinities that were obtained for the pairs of small molecules should therefore be interpreted as relative rankings rather than as absolute binding energies. In addition, solvent effects, beverage pH and the other constituents of each drink were not included in the model. Of these, the pH is likely to be the most significant, since both the structure and the colour of cyanidin-3-glucoside vary with pH. Anthocyanins also occur naturally in beverages such as berry juices and red wine, which may interfere with the indicator's response. Furthermore, GHB showed weak binding

to all of the components tested, suggesting that a separate recognition mechanism will be required for its detection.

Future work should involve immobilising cyanidin-3-glucoside on a paper substrate and evaluating its response in real beverage samples by UV-Vis spectroscopy. Limits of detection should be determined at drug concentrations representative of reported spiking cases, and the screening should be extended to include ketamine and scopolamine. Nevertheless, the computational screening carried out in this study reduced five candidate beverage components to a single lead compound, providing a focused starting point for subsequent experimental validation.

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