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Simulation strategies for characterizing novel phosphodiesterase-5 inhibitors in botanical dietary supplements

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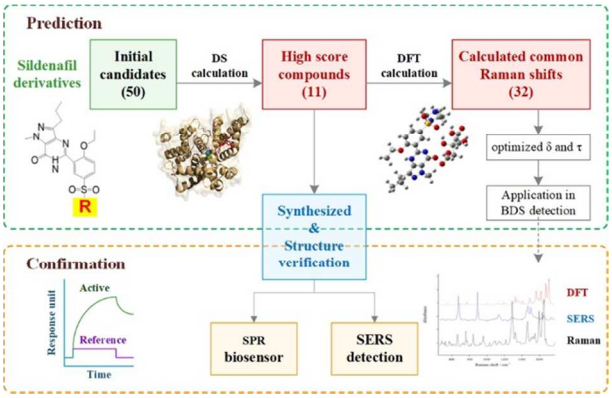
ABSTRACT: A novel "Prediction and Confirmation" (PC) strategy was proposed for characterizing phosphodiesterase-5 inhibitor (PDE-5) derivatives in botanical dietary supplements (BDSs) for on-site detection. Discovery Studio (DS) and density functional theory (DFT) calculations were used for the "Prediction" step in order to estimate PDE-5 derivative structures and theoretical Raman shifts without synthesizing the derivatives. After 11 potentially bioactive sildenafil derivatives were acquired through DS, 32 common calculated Raman shifts were obtained through DFT. The mean absolute wave number deviation (δ, peak range) of the major bands and the minimum number (τ) of Raman spectral peaks matching the calculated common shifts were optimized, so that a positive result of an unknown sample could be reasonably produced. In this study, δ was set at ±10 cm^{-1} and the corresponding τ was set at 4-5 after optimization. Surface plasmon resonance (SPR) biosensor and surface-enhanced Raman scattering (SERS) detection were the "Confirmation" step to validate the reliability and accuracy of DS and DFT in the "Prediction" step, respectively. The optimized δ and τ criteria were used as indexes for on-site SERS detection after thin-layer chromatographic (TLC) separation of six real-world samples, one of which was preliminarily identified as "suspected positive samples." This strategy allows for a quick determination of the BDSs adulterated with sildenafil or its derivatives, independent of any standard materials.



Sildenafil citrate (Viagra®, Pfizer) is a phosphodiesterase-5 (PDE-5) inhibitor used to treat erectile dysfunction (ED) in men. Unfortunately, PDE-5 inhibitors have a negative clinical effect when interacting with compounds including nitroglycerin, doxazosin, and terazosin[1]. As such, botanical dietary supplements (BDSs) that are natural, safe, and free from side effects have successfully captured the market's attention[2]. However, this problem has become increasingly complex as not only synthetic PDE-5 inhibitors, but also structurally modified derivatives of PDE-5 inhibitors, have been found as adulterants. Adulteration with analogues poses a remarkably greater health risk than contamination with approved drugs because of the possibility of adverse effects on cardiovascular function[3].

Many studies have focused on the identification of adulteration using high performance liquid chromatography-mass spectrometry (HPLC-MS), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR). John C. Reepmeyer *et al*[4] detected a new sildenafil analogue in a herbal product and were able to verify its structure through detailed LC-MS and GC-MS. Other adulterated derivatives in BDSs were characterized by Carlo Mustazza *et al*[5], who synthesized PDE-5 inhibitors for detection and structure elucidation by MSMS and NMR. Nonetheless, these technologies are standard-dependent, and the molecular structures of the substances in BDSs are usually unknown. In fact, it is impossible to know or identify all the drugs that could be used as adulterants in BDSs. Even if analysts were aware of what the derivatives might be, there are great difficulties in the synthesis, purification, and standardization of these derivatives. Therefore, there is a need for a new and effective strategy that allows for rapidity, accuracy, portability, and standard-independency.

In our previous study, a thin-layer chromatography and a surface-enhanced Raman scattering (TLC-SERS) method coupled with a common-peak model were developed for the detection of ephedrine and its analogues adulterated in weight loss dietary supplements[1]. Although this method was successful in screening for adulteration with ephedrine and its analogues, it may not be applicable to other kinds of adulterants, such as certain compound's derivatives whose structures are unknown and whose standard materials are unavailable. In fact, it is impossible to predict all derivative structures, or to collect or synthesize all different types of derivatives that may be used as adulterants in BDSs. In this study, we put forward a novel "Prediction and

Confirmation" (PC) strategy for the identification of unknown sildenafil derivatives in BDSs; PDE-5 and sildenafil were selected as the research objects. Discovery Studio 3.0 (DS) was used as the first "Prediction" step for predicting the various derivatives that could be found in BDSs as adulterants. The use of molecular docking in the identification is necessary because it identifies the substituent properties of compounds[6] and provides a rationale for the design of more selective and potent analogues[7]. Density functional theory (DFT) calculation was used as the second "Prediction" step because it serves a relatively efficient and unbiased tool for computing the ground state energy in realistic models of bulk materials and their surfaces [8] and for calculating the molecular vibrational spectra[9]. These two theoretical calculation methods, DS and DFT, were integrated to predict the structure of possible derivative adulterants and their vibrational spectral information.

To verify the effectiveness of the above prediction strategy and to establish an efficient on-site detection method, surface plasmon resonance (SPR) biosensor, which has already become a golden standard in compound-protein interaction determination[10], was used as the first "Confirmation" method for verifying the bioactivity of DS-predicted derivative compounds that were synthesized in our lab. After that, the SERS method was used as the second "Confirmation" step for verifying the correctness of our DFT calculations. Owing to its high sensitivity, SERS is employed after TLC pretreatment of BDSs to detect adulterants whose contents are lower than 1 % (or even 0.1 %)[1,11]. To our best knowledge, there has been no previous report of unknown-derivative detection in the field. In this study, a combination of prediction and detection strategy was proposed, validated, and applied in the determination of sildenafil and its derivatives in BDSs.

EXPERIMENTAL SECTION

Materials and instruments. Sildenafil standard (Code: Y0001578, Europe), tadalafil standard (Code: Y0001417, Europe), and vardenafil standard (Lot: 21127, Germany). Silver nitrate and sodium citrate were purchased from Fisher Scientific. Ultrapure water (18 MΩ cm) was obtained using a Barnstead 1800 filter. The sildenafil derivatives were synthesized by our lab. The BDS products were provided by Shandong Institute for Food and Drug Control (Jinan, China).

A thin chromatographic silica gel 60 F254 layer (10×20 cm) was purchased from Merck (Darmstadt, Germany); ultrasound extraction device (KUDOS-SK5200HP, Shanghai, China), and centrifuge (HERAEUS, FRESCO 17, Thermo Scientific, USA). Separate spots were acquired using an ultraviolet analyzer (WFH-203B, Shanghai Jing Branch Industrial Co., Ltd., China) at 254 nm. Raman spectra were recorded by a portable Raman spectrometer (BWS415, B&W Tek Inc., USA) at 785 nm, at a resolution of 5 cm^{-1} and a 20× long working distance microscope objective.

Molecular docking. We evaluated the reliability of the docking method of PDE-5 inhibitors. The HypoGen module of Accelrys Discovery Studio v3.0 (DS) was used to generate the pharmacophore models. Libdock was used to perform the docking of sildenafil and its derivatives[12]. A high-resolution crystal structure of PDE-5 (PDB code: 1UDT) was downloaded and introduced into DS. All the water molecules were removed from the protein hierarchy and hydrogen atoms were added. The active docking site was defined at 1.41, 66.90, and 83.21 (x, y, z) with a radius of 13. The number of hotspots was set as 100. The docking tolerance was set as 0.25. The other parameters were set as default. Sildenafil and its fifty derivatives were placed at the specific active site receptors, the docking results of which are indicated by a scoring function.

Synthesis of derivatives. The analogues were structurally modified in the piperazine moiety or in the carbonyl of the pyrazolopyrimidine moiety substituted with a thiocarbonyl group[8]. Synthesis started at the intermediate 3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazol[4,3-d]-pyrimidine-5-yl)-4-ethyoxyl-phenylsulfonyl, using chloroform and triethylamine as the solvent and catalyst, respectively. To retain sildenafil derivatives with high activity and considering the feasibility and cost benefits of synthesis, according to the structure-activity relationship of compound structure, the structure of methyl piperazine position on sildenafil was substituted by chemical groups, denoted No. S-102, S-104, S-109, S-110, S-301, S-400, S-401, S-402, S-403, S-404, and S-501. Among the 11 proposed structures, only one has been previously reported. The rest have never been reported as adulterants. The analogues were synthesized starting from a single intermediate, demonstrating that they can be easily synthesized for use as adulterants. The 11 optimized compounds, according to the DS software, are outlined in Figure 1. Their preparation was achieved in a single pathway. In addition, the affinity constants of the ingredients binding to 11 potential targets were determined by SPR assays one by one.

Density functional theory calculation. Vibrational spectra calculations were performed using the Gaussian 09W package of the Linux system[13] with DFT method using the B3LYP hybrid exchange-correlation function[14] and the 6-31G standard (d,p) basis set. The vibrational frequencies were computed at the optimized geometry to ensure that no imaginary frequencies were obtained, confirming that the result corresponds to a local minimum on the potential-energy surface[15].

SPR biosensor analysis. The SPR kinetics of sildenafil and the derivatives were calculated using a Biacore T200 system (GE Healthcare, Sweden). PDE-5 was diluted in 10 mM sodium acetate pH 4.0 and immobilized by the amine coupling method on a CM5 sensor chip with an immobilization level of 7700 RU, according to the manufacturer's instructions. Analytes were diluted in PBS with 5% DMSO running buffer at concentrations ranging from 1 μM to 128 μM with a duplicate middle concentration at the end of each running to confirm the stability of the sensor surface. Analytes were injected at a flow rate of 30 μL/min. The association and dissociation times were 120 s and 300 s. The affinity fitting was carried out using Biacore T200 evaluation software by global fitting with the steady state affinity model.

Sample and silver colloid preparation. The extraction efficiency under fixed ultrasonic extraction conditions was examined in a series of steps. BDS powder (10 mg) was first extracted ultrasonically using 1 mL methanol for 30 min. After centrifugation at 5000 rpm at 4 °C for 10 min, the

residue was removed, and the supernatant was placed in a 10-mL flask and stored at 4 °C for further analysis. The silver colloid was prepared using sodium citrate as the reducing agent, according to the literature[16]. Modified silver colloids were prepared using different solvents, such as glycerol and ultrapure water, of different concentrations (v/v = 1:1)[17].

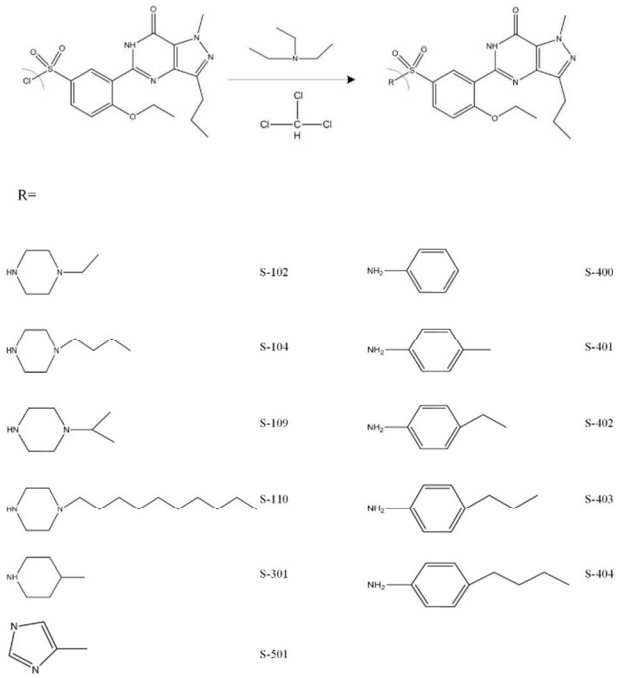


Figure 1. The synthesis pathway of the 11 preferred sildenafil derivatives

TLC-SERS detection method. The TLC-SERS method was designed for on-site detection of adulterant sildenafil analogues in dietary supplements or herbal products claiming to treat erectile dysfunction. First, the 14 compounds (sildenafil, vardenafil, tadalafil, and 11 derivatives) were divided into two groups: (1) vardenafil, tadalafil, S-109, S-403, S-404, S-401, S-501; (2) sildenafil citrate, S-102, S-104, S-110, S-301, S-400, S-402. The two groups (~1 μL) were deposited onto two silica gel TLC plates (10 cm×10 cm, height × weight) loaded at a distance of 10 mm from each other and 1–2 cm from the bottom, respectively. Each plate was developed to a distance of 7.0 cm in a TLC developing chamber previously saturated with dichloromethane: acetone: ammonium hydroxide (20:1.5:0.2, v/v) for 25 min. After the mobile phase evaporated naturally, the separated spots were visualized and marked under ultraviolet illumination at 254 nm (Figure S-1). The SERS signals were then collected with modified silver colloids. High quality SERS spectra were obtained by using a 100 mW laser for 25 s while the spots were still wet. All the measurements were repeated at least three times. The optimization of TLC separation condition could be found in supplementary information.

RESULTS AND DISCUSSION

Molecular docking assays. Considering that sildenafil derivatives with high activity should be produced in a single synthesis pathway, according to the structure-activity relationship of compound structure, a series of 50 sildenafil derivatives were newly designed based on sildenafil's structure. Libdock was used to perform the interplay of sildenafil and its derivatives with PDE-5. The 50 designed sildenafil derivatives were imported into the DS software successively to calculate the theoretical pharmacological action. Eleven high-score sildenafil derivatives were found to have similar pharmacological action and activity intensity as sildenafil (Figure 2). These 11 sildenafil derivatives were then synthesized and characterized by NMR (Bruker AVANCE 600 spectrometer) and an Agilent Technologies 1290 Infinity-6538 UHD Accurate-Mass UPLC-QTOF/MS (Agilent Technologies, Germany) (see in supplementary information), which were chosen for further confirmation study.

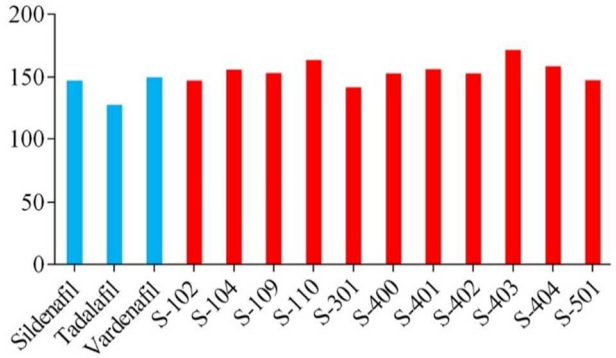


Figure 2. The Libdock score of three post-marking drugs and 11 designed sildenafil derivatives

The docking results of sildenafil and S-102 are shown in Figure 3. The combination of sildenafil citrate with PDE-5 protein resulted in the hydrogen bonding of Gln817 with the nitrogen atom of heterocyclic 1H-pyrazole[4,3-d]pyrimidine . According to the previous studies, Gln817 plays an important role in the interaction between PDE-5 and its inhibitors[18]. Besides, it was found that phenylalanine (Phe820) interacted with heterocyclic 1H-pyrazolo[4,3-d]pyrimidine via π-π interactions. Valine (Val78) also interacted with heterocyclic 1H-pyrazolo[4,3-d]pyrimidine via π-π interactions. Tyrosine (Tyr664), leucine (Leu804), and glycine (Gly819) interacted hydrophobically with the ligands.

The action mode of S-102 is quite similar to that of sildenafil. The combination of S-102 with PDE-5 resulted in the hydrogen bonding of glutamine (Gln817) with the nitrogen atom of heterocyclic 1H-pyrazole[4,3-d]pyrimidine, and the hydrogen bonding of Gln817 with the ketonic oxygen of heterocyclic 1H-pyrazole[4,3-d]pyrimidine. Phe820 interacted with heterocyclic 1H-pyrazolo[4,3-d]pyrimidine via π-π interactions. Val782 interacted with 1H- heterocyclic pyrazolo[4,3-d]pyrimidine via π-π interaction. Aspartic acid (Asn662), serine (Ser663), phenylalanine (Phe786), leucine (Leu725), and alanine (Ala779) were found to interact hydrophobically with the ligands. Other derivatives' docking results are shown in supplementary information (Figure S-2).

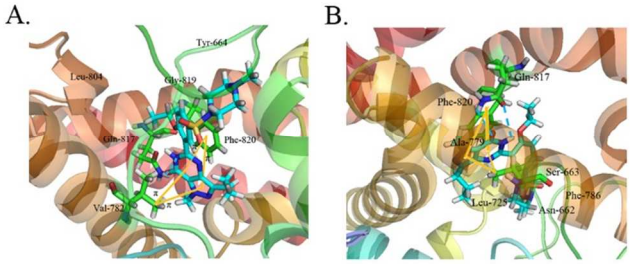


Figure 3. Docking results of sildenafil (A) and S-102 (B).

Interestingly, we found that compound S-102 had the same structure as homosildenafil, an illegally manufactured formulation of adulterated sildenafil that has been found in dietary supplements by Blok-Tip *et al.*[6,19,20]. The simple synthesis of these sildenafil derivatives could be the main reason for their common use as adulterants in botanical dietary supplements, besides homosildenafil. This result indicated that the prediction step used here was accurate and instructive.

SPR biosensor assays. Furthermore, we have performed an SPR biosensor assay using a Biacore T200 system to validate the molecular docking results. SPR biosensor, which is able to monitor the interplay between biomolecules in real-time, is emerging as a powerful tool for biomedical analysis [21]. Currently, the advantage of SPR in the characterization of small molecular ligands specifically bound to target proteins has been recognized[10]. The interactions between synthesized sildenafil derivatives and PDE-5 were characterized by using SPR. The equilibrium dissociation constant (K_d) was calculated, as shown in Figure S-3 and Table S-2. The results of the SPR assays were mostly consistent with the molecular docking results. For example, the highest score of DS was 158.75 for S-404. Similarly, its K_d value was 6.297 μM, which was the lowest among those of all derivatives. The DS prediction was highly credible and can be used to predict the theoretical binding capacity of compounds and proteins.

DFT calculation of sildenafil derivatives. DFT calculation was then carried out on the 11 potential sildenafil derivatives. DFT prediction was performed by using B3LYP DFT, which is implemented in the GAUSSIAN 09 program package[13]. Several basis sets, such as 6-31G (d,p), 6-31+G (2d,p), 6-311++G(3df,pd), cc-pvdz, and cc-pvtz, were utilized. However, since 6-31G(d,p) provided the best compromise between the desired accuracy and computational cost, this basis set was used for the tetramer simulation[22]. The optimized structures of sildenafil and its derivatives are shown in Figure S-4. Based on the DFT results, the 11 high-score sildenafil derivatives from the DS prediction were integrated for comparative analysis.

In this study, 32 common calculated Raman shifts of the 11 optimized sildenafil derivatives have been summarized, namely 443, 652, 688, 717, 751, 765, 782, 809, 827, 849, 891, 927, 940, 976, 1006, 1044, 1050, 1112, 1150, 1174, 1263, 1301, 1322, 1384, 1402, 1428, 1483, 1497, 1532, 1599, 1606, and 1645 cm^{-1}. As shown in Figure 1, S-102, S-104, S-109, and S-110 belonged to the same (aliphatic) class, whereas S-400~404 belonged to another (aromatic) class. Therefore, some of the common peaks belonged to the same compound substituent. For example, the common characteristic peaks of S-102, S-104, S-109, and S-110 at 849 and 1301 cm^{-1} belonged to the ethyl substituent on benzene epoxide; those at 1263 cm^{-1} belonged to the hydrogen atoms on the benzene ring; and those at 1150 cm^{-1} belonged to the methyl substituent on the pyrazole ring. The common characteristic peaks of S-400~404 at 1606 cm^{-1} belonged to the hydrogen atoms on the benzene ring; those at 1150 cm^{-1} belonged to the methyl substituent on the pyrazole ring; and those at 751, 891, and 1263 cm^{-1} belonged to the propyl substituent on the pyrazole ring. Although some of these characteristic peaks could be used as indexes to the piperidine ring substitution and benzene ring substitution, other peaks might be greatly influenced by the neighboring substituent group, intermolecular interaction, or surrounding environment [23], which are usually unpredictable or at least uncontrollable. Consequently, for the on-site detection of unknown derivatives, all 32 common peaks predicted by the DFT theory should be considered to increase the matching rate for the positive judgment.

NRS detection of sildenafil derivatives and common peaks summarization. Because the Normal Raman Scattering (NRS) is more reproducible and accurate than SERS, NRS spectrum was used as the bridging technique for confirming the results of both SERS and the prediction by the DFT calculation in the proposed strategy. We compared the calculated and experimented Raman shifts. When the major band (δ) was set at ± 10 cm^{-1}, 12-20 Raman shifts of the 11 sildenafil derivatives correlated with the above-mentioned 32 common Raman shifts according to the DFT results. When the δ was set at ± 5 cm^{-1}, approximately 6-12 Raman shifts coincided with the above-mentioned 32 common Raman shifts according to the DFT results. When the δ was strictly set at ± 2 cm^{-1}, approximately 1-8 Raman shifts correlated with the above-mentioned 32 common Raman shifts according to the DFT results. As shown in Figure 4, the wider the δ was set, the more Raman shifts will coincide with the DFT prediction. In this study, the NRS results preliminarily confirmed the reliability of the DFT calculations. The 32 calculated common Raman shifts could be used for the preliminary identification in the on-site detection of sildenafil derivatives in BDSs.

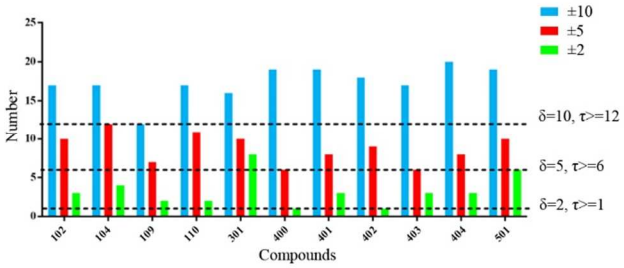


Figure 4. The number of τ with different δ in NRS detection of sildenafil derivatives.

SERS detection of sildenafil derivatives and summarization of common peaks. In the on-site detection of BDS samples, only SERS spectrum was detected after the TLC separation of complex samples, whereas NRS spectrum was inapplicable because the derivative compounds were usually unknown and unavailable, as well as emitting low signal due to low concentrations. Hence, the SERS signal was obtained by using a 100-mW laser for 25 s while the spots were still wet. The optimization of SERS

detection condition could be found in supplementary information (Figure S-5, 6). According to the regulation proposed before, if the δ was set at ± 10 cm^{-1}, S-102, S-104, S-109, S-110, S-301, S-400, S-401, S-402, S-403, S-404, and S-501 would have 8, 22, 13, 13, 8, 10, 15, 10, 15, 10, and 11 matching peaks with the 32 calculated Raman shifts, respectively. If the δ was set at ± 5 cm^{-1}, these compounds would have 6, 14, 5, 9, 5, 6, 9, 8, 6, 4, and 5 matching peaks, respectively. If the δ was set at ± 2 cm^{-1}, the compounds would have 1, 5, 1, 2, 1, 0, 1, 3, 4, 2, and 2 matching peaks, respectively. According to these results, the δ value of 2 is an overly strict standard that caused some samples to have only one or even no matching peak. Hence, this setting was not used in the subsequent study. Suppose that δ was set at ± 10 cm^{-1}, the 11 derivatives would then contain at least 8 of the calculated common Raman shifts. If δ was set at ± 5 cm^{-1}, then the 11 derivatives would contain at least 4 Raman shifts. In this study, we used a δ value of ± 10 cm^{-1}.

Detection of simulated samples by TLC-SERS. In order to show the feasibility of the developed TLC-SERS method, 10 simulated samples were separated and detected by using the established TLC-SERS method. After TLC separation, the prepared silver colloid was dropped on the separated spot which was then visualized under 254 nm (Figure S-7). According to the common calculated peaks, sample 19 exhibited the SERS signal at 419, 484, 553, 630, 658, 717, 904, 926, 1098, 1259, 1293, 1312, 1560, and 1583 cm^{-1} for the first spot. When δ was set at 10 cm^{-1}, the results of the detection contained 6 Raman shifts of the calculated common shifts. When δ was set at 5 cm^{-1}, the results contained 3 Raman shifts of the calculated common shifts. The SERS signal was found to be 485, 814, 909, 1004, 1109, 1025, and 1396 cm^{-1} for the second spot. With δ set at 10 cm^{-1}, the results of the detection contained 4 Raman shifts of the calculated common shifts. With δ set at 5 cm^{-1}, results of the detection contained 3 Raman shifts of the calculated common shifts. The SERS signal was found to be 525, 570, 733, 753, 910, 948, 1040, 1096, 1236, 1266, 1530, and 1562 cm^{-1} for the third spot. With δ set at 10 cm^{-1}, the results of the detection contained 5 Raman shifts of the calculated common shifts. With δ set at 5 cm^{-1}, the results of the detection contained 4 Raman shifts of the calculated common shifts. The SERS signal was found to be 418, 484, 535, 819, 926, 990, 1009, 1185, 1236, 1332, and 1529 cm^{-1} for the fourth spot. With δ set at 10 cm^{-1}, the results of the detection contained 5 Raman shifts of the calculated common shifts. With δ set at 5 cm^{-1}, the results of the detection contained 3 Raman shifts of the calculated common shifts. The results showed that the doping substrate was sildenafil or one of its derivatives. Furthermore, the first spot was found to be at the same position as S-109 on the TLC plate, suggesting that S-109 was adulterated in this sample. The remaining spots were found to correspond to S-301, S-400, and S-501, respectively (Figure S-8). Because the concentration of the derivatives was very low and the Raman enhancement was irregular on the TLC stationary phase (silica particles), some of the Raman peaks were not enhanced and would sometimes even disappear in the SERS spectrum, which resulted in the loss of available common-peak information. In addition, the differences in instrument and sample status would inevitably lead to the fluctuation of Raman shifts. Hence, the number of detected peaks in the simulated samples was lower than in the standard samples. When δ = 10, τ decreased from 8 (standard samples) to 4 (simulated samples); when δ = 5, τ decreased from 4 (standard samples) to 3 (simulated samples). These results confirmed that setting the δ at ±10 cm^{-1} was appropriate for the simulated sample. I.e., a positive result was obtained if the sample contained at least 4-5 Raman shifts of the calculated common shifts.

Detection of real samples by TLC-SERS. Furthermore, the method was applied to detect real samples. Six real samples were provided by the Shandong Institute for Food and Drug Control and analyzed by using TLC-SERS. The results were based on the 32 common calculated peaks and the δ was set at ± 10 cm^{-1}. Because these samples contained at least 4-5 Raman shifts of the calculated common shifts, they were preliminarily identified as "suspected positive samples" for further verification in the lab. Sample 5 displayed a Raman signal at 487, 559, 736, 745, 807, 816, 916, 927, 1042, 1098, 1169, 1180, 1229, 1237, 1316, 1529, 1559, and 1584 cm^{-1}. Sample 5 contained **7** Raman shifts that matched the calculated common shifts. This sample was identified as a sildenafil derivative adulterant. After comparison with the Raman shifts of 11 sildenafil derivatives, sample 5 was found to be doped with S-109 (Figure 5). The TLC-SERS results were further confirmed by using UPLC-QTOF/MS (Figure S-9). The result indicated that the 32 calculated common peaks can be used as a measure to discriminate suspected adulterants in BDSs. Through the validation of suspected positive samples using UPLC-QTOF/MS, PC strategy was found to be able to quickly and accurately detect sildenafil derivatives in BDSs. Generally, if the τ is fixed, the reduction of δ value will result in an increased false-negative rate and the rise of δ value will result in an increased false positive rate. It is worth noting that screening or identification based on one specific Raman peak was insufficient to produce a definitive result; thus, identification using a series of Raman peaks (at least four or five peaks unique to each synthetic drug) was necessary for screening purposes[11]. According to the previous results, δ should be set at ± 10 cm^{-1} for on-site detection. If the tested BDSs had been collected from a relatively reliable sales channel, τ should be set at 4-5. On the contrary, if the tested BDSs had been collected from suspicious or unknown sales channels or had a record of adulteration, the value of τ should be stricter to reduce the false-negative rate. In order to set the appropriate value of τ, we referred to other SERS-based methods. In the detection of melamine in milk samples, as long as one Raman shift at 675 cm^{-1} was detected in a sample, that sample was suspected to be adulterated with melamine[24,25]. As for the detection of sildenafil and its derivatives, τ should be set at 2. In other words, if 2 common shifts were identified in a sample from suspicious or unknown sales channels or with a record of adulteration (as in the case of melamine-milk), this sample would be regarded as a suspicious sample.

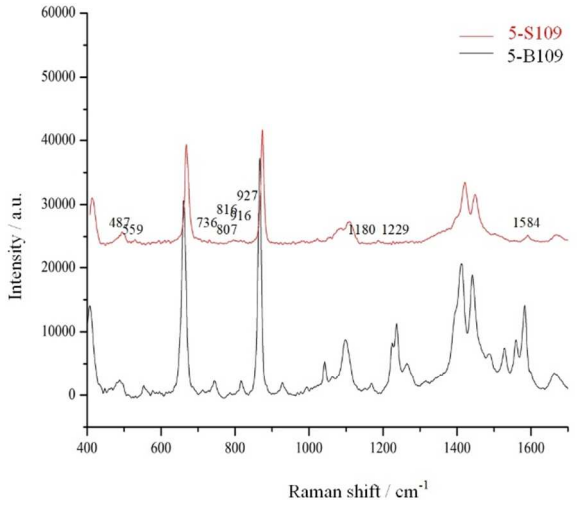


Figure 5. TLC-SERS results of sample 5.

During its construction, the procedure of PC strategy was, in fact, quite complex because it needed various confirmation approaches, including compound synthesis, structure validation, and SPR assay, to verify the accuracy of the prediction results. However, once the PC strategy was successfully constructed, complex confirmation steps can be omitted from the on-site detection. It was possible to judge whether a sample contains sildenafil and its derivatives directly through TLC-SERS using optimized δ and τ with no need of any standard materials. Our proposed method was more conforming to the requirement of on-site detections than existing methods.

CONCLUSION

Computer-assisted drug designing was carried out to simulate compounds with target enzymes for calculating the target compounds and evaluating their corresponding activity. The results of the DFT calculation directly reflected the molecular vibrational spectra of each compound. Combining the two theoretical calculations, DS and DFT, in the absence of a series of derivatives of the PDE-5 inhibitors, allowed us to predict their bioactivity and theoretical Raman spectra. Sildenafil and its derivatives were identified as adulterants in BDSs through on-site SERS detection with optimized δ and τ. The comprehensive, quick, and accurate PC strategy helped us resolve the lack of derivative standards. The PC strategy was also suitable for comprehensive, quick, and accurate detection of other PDE-5 inhibitors, such as tadalafil and vardenafil, based on the differences in their structures.

ASSOCIATED CONTENT

Supplementary Information

Supporting figures include UPLC-QTOF-MS analysis and NMR analysis of S-102, S-104, S-109, S-110, S-301, S-400, S-401, S-402, S-403, S-404 and S-501, TLC separation of reference substances (Figure S-1), identification of sildenafil analogues (Table S-1), molecular docking results (Figure S-2), SPR affinity assay results (Figure S-3 and Table S-2), DFT results(Figure S-4), selection of SERS detection condition (Figure S-5), SERS detection results of the sildenafil derivatives (Figure S-6), TLC results of the simulated samples (Figure S-7), comparison of TLC-SERS and Raman results of the simulated samples (Figure S-8), UPLC-QTOF/MS results of sample 5 (Figure S-9). The Supplementary Information is available free of charge on the ACS Publications website.

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Notes

The authors declare no competing financial interest.

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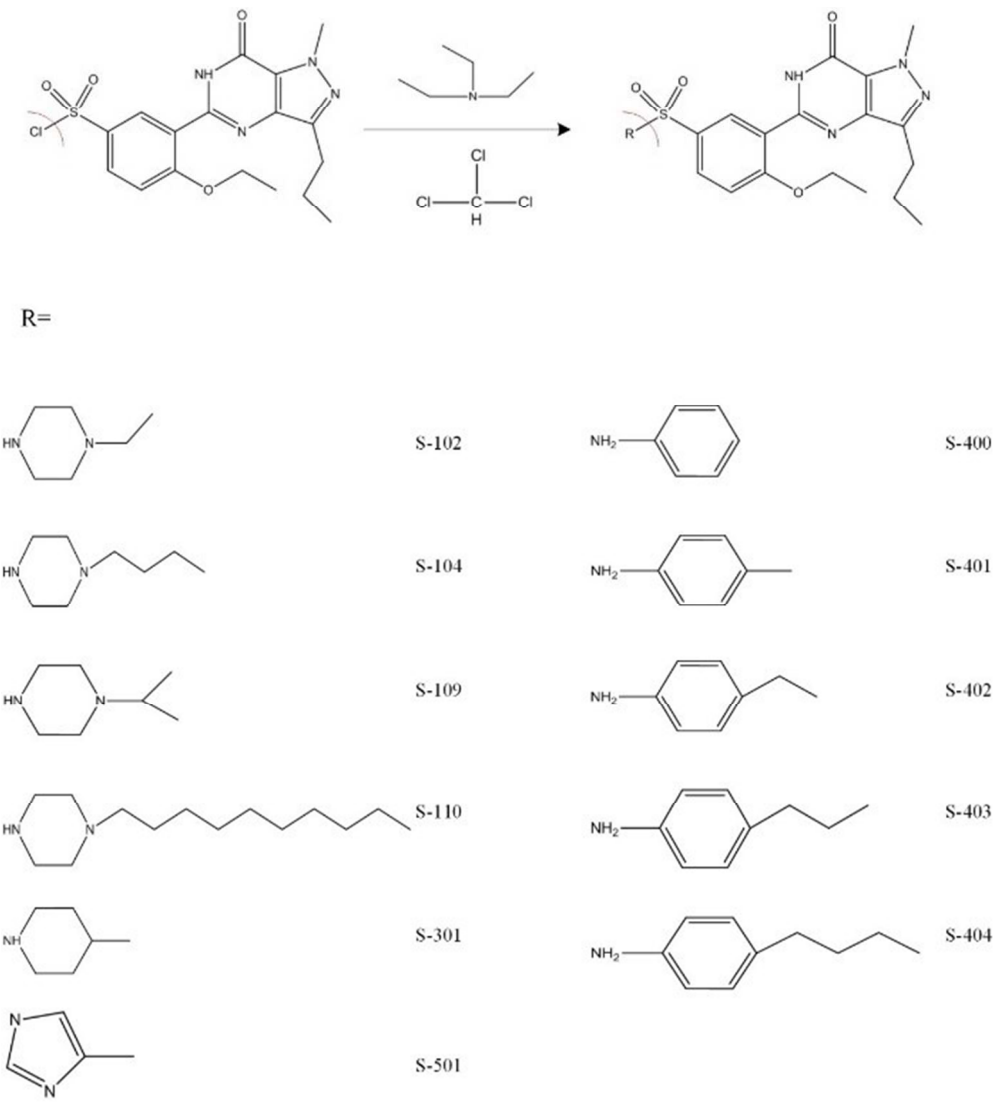

Figure 1. The synthesis pathway of the 11 preferred sildenafil derivatives

81x88mm (220 x 220 DPI)

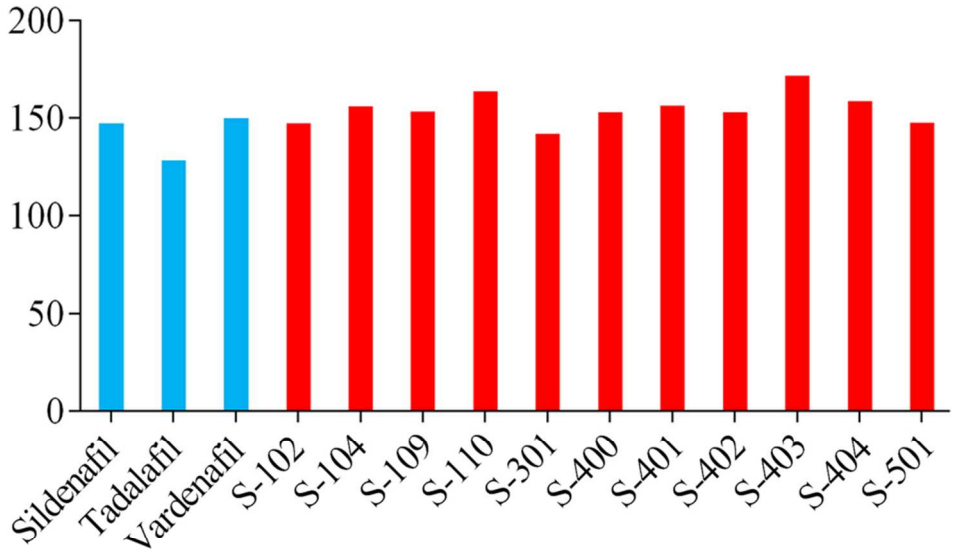

Figure 2. The Libdock score of three post-marking drugs and 11 designed sildenafil derivatives

85x57mm (300 x 300 DPI)

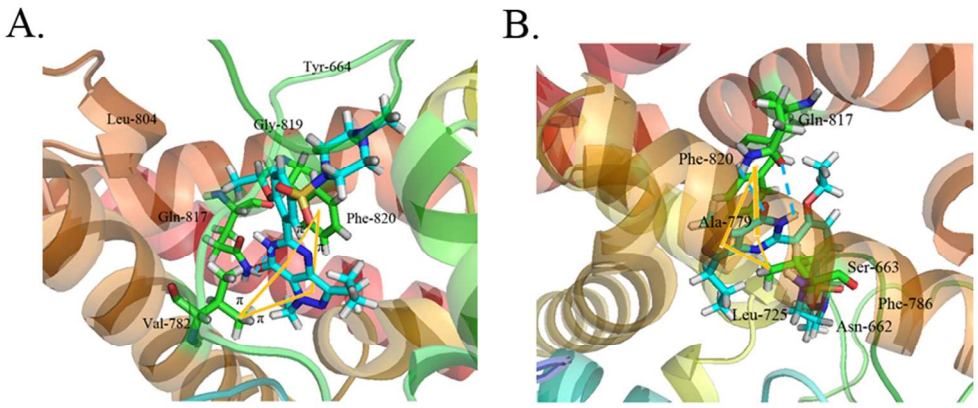

Figure 3. Docking results of sildenafil (A) and S-102 (B).

85x38mm (300 x 300 DPI)

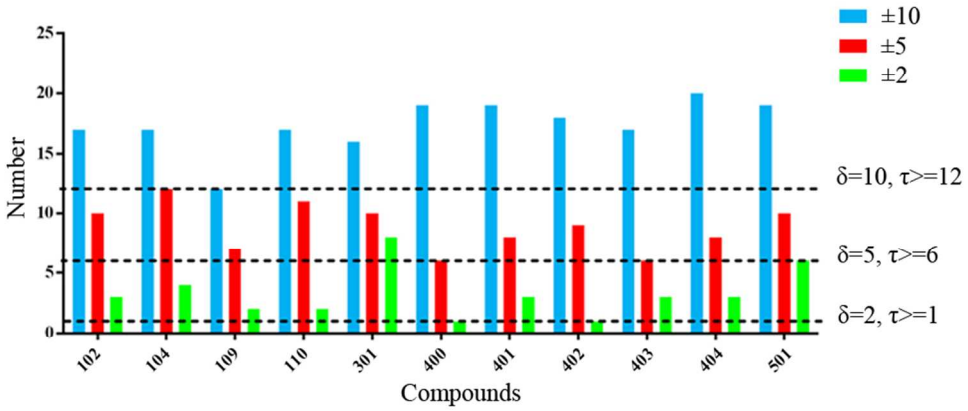

Figure 4. The number of τ with different δ in NRS detection of sildenafil derivatives.

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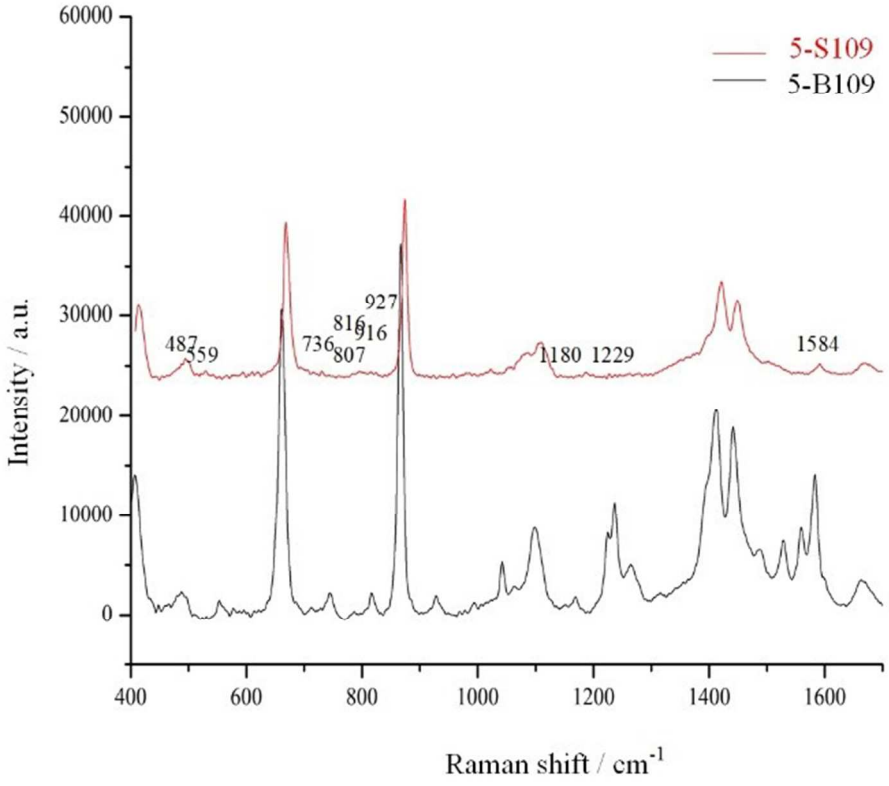
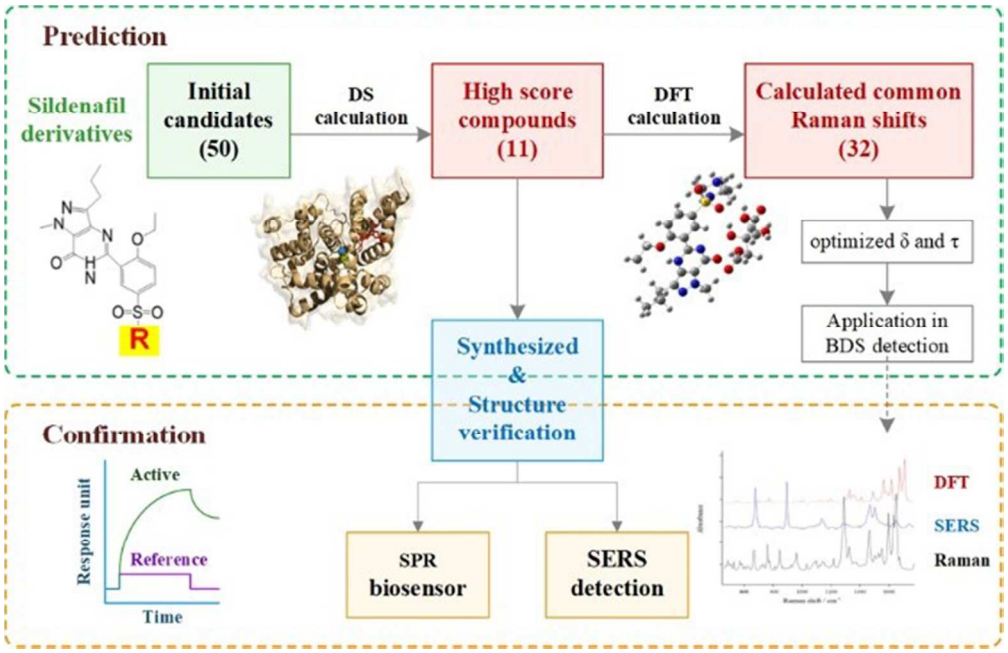


Figure 5. TLC-SERS results of sample 5.

84x80mm (220 x 220 DPI)



80x51mm (220 x 220 DPI)