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A rapid detection method for on-site screening of estazolam in beverages with Au@Ag core-shell nanoparticles paper-based SERS substrate

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Abstract

Estazolam (EST) is a common sedative-hypnotic drug with a risk of abuse. Therefore, rapid on-site detection of EST is necessary to control the abuse of EST. In this paper, a fast, simple, and sensitive method is demonstrated for the detection of EST in both water and beverages that use surface-enhanced Raman spectroscopy (SERS) techniques. Au @Ag core-shell nanoparticles (NPs) assembled on the filter paper as a SERS substrate exhibit good applicability and practicality. At the same time, density functional theory (DFT) is used to assign the vibration mode of the EST molecules, which guides for subsequent experiments. The lowest detectable concentration of EST in aqueous solution can be as low as 5 mg/L, and signal uniformity is excellent (RSD_{500} = 5.56%, RSD_{1000} = 4.35%). In addition, EST components artificially added to orange juice and pomegranate juice can be effectively detected by simple pretreatment with a minimum detection concentration as low as 10 mg/L. Therefore, the use of Au@Ag core-shell nanoparticles paper-based SERS substrate provides a quick and easy method for the detection of illegally added drugs in beverages.

Keywords: SERS, Estazolam, Au @Ag core-shell NPs, density functional theory, Dilution pretreatment, beverages
Introduction

Benzodiazepine estazolam (EST) is a potent, hypnotic prescription that is often used to treat insomnia.1-3 According to China’s national administrative regulations, illegal sales of excessive EST are equivalent to drug trafficking. Additionally, reports of cases on the use of EST for drug-induced sexual assaults still occur from time to time. In the previous literature reports, there are many methods for detecting estazolam, such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Liquid Chromatography-Mass Spectrometry (LC-MS).4-7 These methods can achieve excellent detection concentrations. However, the pre-conditions such as complicated pre-processing steps, long detection time and the unportable instrument cannot meet the requirements of real-time on-site detection. Therefore, it is of considerable significance to study a rapid, sensitive and reliable EST field detection method.

Surface-enhanced Raman spectroscopy (SERS) has been widely used in land testing, medical diagnostics, food safety, environmental monitoring and other fields.8-10 As a new detection method based on Raman spectroscopy, it not only overcomes the shortcoming of substantial fluorescence interference in traditional Raman detection but also dramatically enhances the Raman signal due to the local surface electromagnetic field resonance of the rough precious metal surface.11,12 At the same time, the pretreatment of SERS is simple. The spectrum acquisition speed is fast and the spectrometer is portable, which satisfies the on-site and real-time sample detection requirements.13-15

In recent years, SERS has gradually emerged in the cross-disciplinary field of food and drug, and research on benzodiazepines has made some progress. However, the use of SERS for qualitative and quantitative detection of EST still lacks systematic research in the Raman spectral library. The vibration mode attribution of EST in the Raman spectral library needs to be improved, and verification will be needed to confirm whether SERS technology can effectively detect EST in beverages containing EST tablets. Therefore, in this article, we have studied these aspects. At the same time, in order to pursue the stability and repetition rate of the test, we also adjusted the SERS substrate. Traditionally, gold and silver nanoparticles have been the first choice for SERS detection.16-18 AuNPs have better biocompatibility, long-term stability, and optical response, while Ag NPs have stronger surface plasmon resonance (SPR).19-20 In order to combine the advantages of Au and Ag, we prepared Au-Ag bimetallic NPs with a core-shell structure. For this structure, although Ag+ is tightly packed on the surface of Au NPs, the actual distribution of Au core and the Ag shell is separated.21 Theoretically, a bimetallic Au@Ag NP can provide a better optical response.22-24 For ease of storage and application, we also used Langmuir technology to assemble Au@Ag NPs onto filter paper for use as the final SERS detection substrate.25-27

In this paper, a novel method for the detection of EST using the paper-based SERS substrate decorated by the core-shell Au@Ag NPs was proposed. By analyzing the characteristic bands of the Raman spectra, the structural information of the EST molecule could be obtained, which provided the basis for the analysis of the later EST medicine. Moreover, a standard curve between the intensity and concentration of the EST spectrum is established, which provides a reference for the quantitative detection of EST. In addition, as proof for practical application feasibility to actual samples, we analyzed two beverage samples spiked with EST using dilution as a simple pretreatment procedure.

Experimental

Reagents and chemicals

Silver nitrate, dichloromethane (DCM; CH₂Cl₂), were offered by the Sinopharm Chemical Reagent Co., Ltd. The L(+)−ascorbic acid (C₆H₇O₆) was purchased from Alfa Aesar (China) Chemical Reagent Co., Ltd. Cetyltrimethylammonium bromide (CTAB) and polyvinyl pyrrolidone (PVP) were purchased from Sigma-Aldrich Co., Ltd., Sodium borohydride (NaBH₄) was purchased from Acros Organics Co., Ltd. Cetyltrimethylammonium chloride (CTAC, 97%) and Malachite green (MG) were obtained from Aladdin Industrial Corporation. EST standard material was offered by National Institutes for Food and Drug Control and tablets of EST were offered by Heilongjiang first specialized hospital. None of the chemicals were further purified. Milli-Q water (18.0 MΩ•cm) was purified with a Sartorius Arium 611 UV ultrapure water system.

Preparation of Au@Ag nanoparticles

AuNPs were prepared by a seed-mediated growth method following the established methods proposed by Xia’s group,28 the average particle diameters were about 46nm. Then, the Au@Ag core-shell NPs were synthesized by depositing Ag+ on the surface of Au NPs reference to Peiyan Yuan’s method with
some adjustments. Simply said, AgNO$_3$ solution (10 mM) of 0.25 mL and 0.3 mL AA solution (100 mM) were mixed with Au NPs seed solution (10 mL) which is re-dispersed into the same volume of 80 mM CTAC in a 20 ml vial under vigorous stirring room temperature for 2 minutes, and then gently stirred at 60 °C for 1 hour. After that, the reaction was stopped by an ice-water bath, and the product was centrifuged and dispersed in a CTAC solution (20 mM).

**Self-assembly of filter paper substrates**

The assembly process can be referred to in our previous work. The phase transfer was carried out using the PVP ethanol solution as the intermediate carrier. 10 ml prepared Au@Ag core-shell NPs were centrifuged (2000 × g 10 min) and dispersed in the same amount of 1 wt % PVP in ethanol. Then the washing dispersed Au@Ag NPs which were recentrifuged (2100 × g 10 min) were transferred to ethanol and placed in a 10 ml centrifuge tube. Subsequently, a small amount of trichloromethane, the same volume mixed with n-hexane, and a few drops of water were added into this test tube. that the tube was shake violently for one minute and then left standing still for about 2 minutes. A layer of dense nanometer Au@Ag gold monolayer film appeared between the oil and water interface. The nanoparticle monolayer film was then taken out using filter paper as a carrier material for later testing.

**Sample preparation**

An EST aqueous solution with a series concentration of 100, 75, 50, 25, 10, 7.5, 5, 1 mg/L were prepared using EST standard substance by ultra-pure water. However, common EST is in tablet form, which contains many auxiliary materials and 1mg EST. Therefore, it is necessary to extract the main components of EST from other auxiliary materials. Here, the extraction method of the main components in the tablet refers to the work in other literature. According to the dissolution curve of EST and its solubility in water, EST needs to be fully dissolved at a specific temperature and time. The process included first grinding the tablet, then dissolving the drug powder in 10 ml of beverage at 37 °C for 30 minutes. Next, the precipitation was removed by 5000 × g centrifugation for 5 minutes. The estimated concentration of this purified solution is 100 mg/L, and then this purified solution was diluted with the beverage to obtain the actual samples with different concentrations of 75, 50, 25, 10, 5 and 1 mg/L.

**SERS measurements**

The filter paper substrates were immersed in the sample solution for 5 min, after that, take the filter paper out and lay it on the objective table. The laser probe working distance is 6.9 mm, with an excitation light wavelength at 785 nm, focusing on the center of the filter paper substrates. Then, the collected Raman spectra were recorded by a portable Raman spectrometer BWS415-785h (B&W Tek, Inc.) with a spectral range of 175-2000 cm$^{-1}$ and a resolution better than 3 cm$^{-1}$. The output power is maintained at 25% (75 mM) and the integration time is 5 s. Boxcar averaging was used to smooth the spectra, and the software background corrected the data.

**Computational details**

Gaussian09 (Gaussian, Inc.) was used for all calculations. Gas-phase of the EST molecule is geometrically optimized and the vibrational spectrum is calculated by using the B3LYP exchange-correlation function and standard 6-31g (d) basis set. The Gaussview05 program provides a visual animation and completes the normal mode assignment.

**Results and Discussion**

**Characterization of NPs**

The morphology and particle size distribution of nanoparticles are the main factors affecting their properties, which are controlled by process conditions. In this paper, on one hand, the morphology of the Au NSs and Au@Ag NPs were characterized through scanning electron microscopy (SEM), ultraviolet and visible spectrophotometer(UV/Vis) and dynamic light scattering (DLS), on the other hand, the core-shell structure of Au@Ag NPs was characterized through transmission electron microscope (TEM). Monodisperse Au nanospheres were fabricated according to the seed growth method in the aqueous phase, and the SEM has shown in Fig. 1a. Meanwhile, due to the matched crystalline lattices between Ag and Au, Ag$^+$ was tightly deposited on the surface of the Au spheres by a reduction reaction, forming the approximate spherical shell, as shown in Fig. 1b. Then, the size distribution calculated is shown in Fig. 1c. The average diameters for the Au NSs are 45.3 ± 5.4 nm (~46 nm) and for Au@Ag NPs are 60.5 ± 7.8 nm (~60 nm) with a Gaussian distribution,
respectively. Furthermore, in Fig. 1d, the extinction spectrum of Au NSs has a blue-shift after coating with Ag shells and the macroscopic states of the nanoparticles were changed from pink to orange. From the results of the characterization, the Au @ Ag NP has good particle size uniformity and monodispersity.

The properties of the paper-based substrate on which the nanoparticles are assembled were characterized by SEM. As can be seen in Fig. 2a, b, c, after self-assembly through oil/water interface technology, a tightly arranged single layer Au@Ag NPs can be attached to the fiber surface of filter paper substrate. The pore structure on the fiber surface provides a larger surface area to attach more plasma nanomaterials, while the tightly arranged nanoparticles provide a stable enhancement effect. Then, we used 1 μM MG (target molecule) aqueous solutions to demonstrate its applicability. SERS spectra of 25 arbitrary spots on the paper-based substrate were collected. (Fig. 2d) The signal intensities from different spots of MG (1614 cm\(^{-1}\)) with relative standard deviations (RSD) of 7.04 % are shown in Fig. 2e. The results show that the Au@Ag NPs paper-based SERS substrate can provide a stable and repeatable detection environment of SERS.

**Raman Spectra of EST and its attribution**

The accurate Raman peak assignment of EST was rarely reported before this study, and most literature provides only a small fraction of the Raman displacement, or only speculates that the position of a peak is added. Therefore, DFT calculation was applied to assign the vibrational bands of EST molecular. The B3LYP function and 6-31g (d, p) basis in gauss 09 were finally determined by comparing the calculated results with the measured spectra. According to the scale factor database of vibration frequency, 0.991 was selected as the correction factor.

The optimized EST structure is shown in Fig. 3. The observed SERS spectra of EST aqueous solution and the theoretically calculated EST spectra are shown in Fig. 4. By comparing the two spectra, the strongest predicted Raman spectra are in good agreement with the experimental results, but there are some slight differences. This may be due to different external conditions. EST Raman spectrum is simulated under vacuum conditions, and in contrast, the SERS spectrum was obtained in the water phase by molecular adsorption on silver-coated gold nanoparticles.

The vibration analysis results are shown in Tab.1. Most of the medium and strong spectral bands that appeared in the EST SERS spectrum also appeared in the calculated spectrum, and the position of the spectral bands did not change much. For example, the strongest bands at 1000 cm\(^{-1}\) that appeared in the EST SERS spectrum also appeared in the calculated spectrum, and the position of the band is added.

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The vibration analysis results are shown in Tab.1. Most of the medium and strong spectral bands that appeared in the EST SERS spectrum also appeared in the calculated spectrum, and the position of the spectral bands did not change much. For example, the strongest bands at 1000 cm\(^{-1}\) are caused by respiratory vibrations of Ring1, Ring3, and C\(_6\)N\(_{10}\). The second strongest Raman band is located at 687 cm\(^{-1}\), due to the bending vibration mode of Ring4. The medium Raman band of 1166 cm\(^{-1}\) was caused by the stretching vibration of the benzene diazine heterocyclic ring (Ring1, Ring2), and the breathing vibration of Ring1 caused the Raman band of 1606 cm\(^{-1}\). Other moderating and weak peaks are listed in Table 1.

**Determination of EST in aqueous solution by SERS**

The Aqueous solution is the most common carrier of medicine. Consequently, it is the most basic starting point to detect EST in aqueous solution and provides the reference for the follow-up detection. The SERS spectrum of EST solution with different concentrations was shown in Fig. 5a, and the main vibration bands of EST at 687 cm\(^{-1}\), 1000 cm\(^{-1}\) repeatedly showed that the spectral intensity decreased with the degressive concentrations. It can be seen from the figure that when the concentration was reduced from 5 mg/L to 1 mg/L, other vibration bands appeared due to the enhanced background interference and competition mechanism. Fortunately, there were differences between the background spectra and the EST SERS spectra, which can be distinguished by the vibration bands of 687 cm\(^{-1}\) and 1000 cm\(^{-1}\). The bands at 687 cm\(^{-1}\) and 1000 cm\(^{-1}\) had a steep peak distribution observable and the sample concentration was then statistically analyzed (Fig. 5b). The regression curve which corresponds to the band at 687 cm\(^{-1}\) was obtained via regression analysis y=14085.62-13166.28×exp[-x/124.34] and the fitting factor was as high as R\(^2\)=0.998. At the same time, the regression curve of the band at 1000 cm\(^{-1}\) was y=12176.57-11703.38×exp[-x/44.36] and the fitting factor was R\(^2\)=0.996. According to the correlation coefficient, the fitting curves of Raman signal intensity and sample concentrations have a high fitting degree.

As shown in the graph, the characteristic bands at 687 cm\(^{-1}\), 1000 cm\(^{-1}\) with 1 mg/L EST solution can be clearly seen. Through calculation, the recognition condition that signal-to-noise-ration is not less than 3 dB is satisfied. Hence, it could be regarded as the minimum detected concentration, which has practical significance.
The repeatability of the SERS method for detecting signals has always been an important parameter. In this experiment, to verify the reliability of the experimental results, 25 groups of tests were collected in 50 mg/L of an EST aqueous solution, as shown in Fig. 6a. In addition, the spectral intensity of the characteristic bands at 1000 cm\(^{-1}\) and 687 cm\(^{-1}\) was statistically analyzed, as shown in Fig. 6b (each group was measured 5 times and the points in the graph are averages). The RSD\(_{687}\) was 5.56% and the RSD\(_{1000}\) was 4.35%, which indicated that this test method had good repeatability and stability.

According to the fitting curve, the recovery rates of samples with configured concentrations of 7.5 mg/L and 75 mg/L were calculated, and the calculated results are shown in Table S1 (Supporting Information). Although SERS is a fast detection method, the fluctuation of the Raman signal cannot be ignored. These fluctuations are mainly caused by the difference in adsorption between nanoparticles and molecules. Environmental factors and experimental errors also have a certain impact on the fluctuations. Therefore, we selected a solid substrate assembled on the filter paper, selected the composite Au@Ag nanoparticles as the nano substrate to make the particle size more uniform, and then we adjusted the laser acquisition time to 5 s to provide a relatively stable spectrum. These factors together provided better signal reproducibility for the SERS method.

**Determination of EST in simulated real sample by SERS**

The ability to detect actual samples is an essential basis for SERS technology for practical application testing. Therefore, we conducted an experimental study on two juice drinks mixed with estazolam tablets. \(^{[28]}\) However, due to the complexity and diversity of the beverage sample components, the interference of the original components in the beverage to the rapid detection cannot be ignored. Therefore, it is necessary to perform pretreatment on the actual sample before the detection process to improve the detection efficiency and the intensity of the SERS signal. In the preliminary experimental exploration, we found that the SERS signal collected by diluting the actual sample with 1.5 volume of water is superior to the SERS signal collected directly. This phenomenon may be because other ingredients in the beverage interfere with the adsorption of drug molecules on the surface of nanoparticles, thereby reducing the enhancing effect of nanoparticles on the surface of molecules.

When the diluted solvent was closer to the aqueous solution, the interference from the solvent was reduced and the SERS signal was enhanced. However, at the same time, as the dilution is excessive, the drug concentration is also lowered, and the SERS signal eventually tends to decrease. It should be noted here that the concentration of EST in the diluted beverage has changed, and the lowest concentration detected below refers to the concentration before dilution. The correspondence between the concentrations before and after dilution is shown in Table 2.

The detection of actual samples ultimately led to a strategy for the simultaneous dilution of beverages and drugs. After dilution, we detected the pre-processed simulated real samples, and the results are shown in Fig. 7. In the simulated sample solution, the characteristic bands of EST at 687 cm\(^{-1}\), 1000 cm\(^{-1}\) can be clearly detected when the concentration is about 10 mg/L. In addition, we found that in the actual samples, the relationship between concentration and intensity is not strictly consistent with the fitted curve in the aqueous solution. This result may be due to interference from some external factors such as the detection environment. When the concentration of the drug and the intensity of the characteristic band are very low, the influence of external factors becomes more obvious, which has a certain influence on the accuracy of the test results. At the same time, according to the guidelines of pharmaceutical companies, the correct dose of sleeping pills EST for sleep disorders is 1-2 mg. In the case of illegal abuse of psychotropic substances in general, criminals often incorporate overdose drugs into beverages to deprive victims of their consciousness. \(^{[46]}\) Therefore, the lowest concentration detected is 10 mg/L, which meets the requirements for on-site screening. The result also indicates that the rapid detection of EST in beverages and aqueous solutions undoubtedly has broad potential in practical application.

**Conclusions**

In this study, the detection substrate of this method is Au@Ag nanoparticles compounded on filter paper. The uniform particle size of the nanoparticles, monodispersity, and stability of the solid filter paper material make the substrate easier to use and store. Then, this solid substrate was used for the detection of psychotropic EST, and the result shows that EST added in water or beverages could be quickly identified. On detection, firstly, the density functional theory is used to assign the vibration mode in detail to guide the subsequent specific experimental analysis. Secondly, the qualitative and semi-quantitative analysis of psychotropic EST was carried out. The equation at 687 cm\(^{-1}\) and 1000 cm\(^{-1}\) is: 

\[
\text{EST (mg/L)} = \text{SERS intensity at 687 cm}^{-1} - \text{SERS intensity at 1000 cm}^{-1}
\]

This equation is used to calculate the concentration of EST in the sample. The corresponding concentration range is 0-50 mg/L, which meets the requirements for on-site screening. Therefore, this method is suitable for the rapid detection of psychotropic EST in beverages and aqueous solutions. It is hoped that this work can provide a new method for the rapid detection of psychotropic substances in beverages and contribute to the prevention and control of drug abuse.
cm\(^{-1}\) was calculated, according to the relationship between the concentration of multiple points and the Raman intensity. The fitting curve equations were \(y=14085.62-13166.28\times\exp[-x/124.34]\) and \(y=12176.57-11703.38\times\exp[-x/44.36]\). At the same time, based on the fitting curve, the recovery rates of two low and medium concentrations randomly selected were verified, and the minimum detected concentration reached 5 mg/L by experiments. Thirdly, to study the practical application value of this method, EST tablets were added to orange juice and pomegranate juice for testing, and the results showed that the beverage would interfere with the Raman signal. Finally, in order to reduce the interference, we introduced the dilution method here as a simple and necessary pretreatment step, and the minimum detected concentration of EST in two beverages reached 10 mg/L after pretreatment. In conclusion, this method is fast, accurate, non-destructive, and easy to operate. It has good application potential and practical value in drug abuse detection.

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**Supporting Information**

**Table S1. Analytical result for EZM in aqueous solution**

| Spiked (mg/L) | Sample | Band at 1000 cm\(^{-1}\) Intensity (a.u.) | Found (mg/L) | Recovery % | Band at 687 cm\(^{-1}\) Intensity (a.u.) | Found (mg/L) | Recovery % |
|---------------|--------|------------------------------------------|--------------|------------|------------------------------------------|--------------|------------|
| 5             | 1      | 1769.846                                 | 5.208        | 104.1      | 1562.407                                 | 6.226        | 124.5      |
|               | 2      | 1812.203                                 | 5.389        | 107.7      | 1524.589                                 | 5.851        | 117.0      |
|               | 3      | 1897.996                                 | 5.758        | 115.1      | 1546.044                                 | 6.063        | 121.2      |
|               | Average | 5.451                                    | 108.9        |            | 6.046                                    | 120.9        |            |
| 75            | 1      | 9799.959                                 | 73.358       | 97.8       | 6456.078                                 | 67.843       | 90.4       |
|               | 2      | 10036.91                                 | 75.377       | 100.5      | 6661.794                                 | 71.242       | 94.9       |
|               | 3      | 11750.35                                 | 93.37        | 124.4      | 7105.953                                 | 78.913       | 105.2      |
|               | Average | 80.701                                   | 107.5        |            | 72.666                                   | 96.8         |            |

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Table 1. Theoretical and experimental vibration frequencies of EZM

| Theoretical Calculations/cm⁻¹ | SERS/cm⁻¹ | Adscription                      |
|-------------------------------|-----------|----------------------------------|
| 683                           | 687 s     | δ(Ring4)                         |
| 755                           | 759 w     | δ(Ring1) + δ(Ring3) + δ(N₂-C₉=N₄) |
| 790                           | 787 w     | δ(Ring1) + ν(Ring2) + δ(Ring4)   |
| 942                           | 935 w     | δ(Ring3) + δ(Ring1)              |
| 977                           | 980 w     | δ(Ring3)                         |
| 1004                          | 1000 s    | ν(Ring1) + δ(Ring3) + δ(C₉-C₁₀-N₃) |
| 1034                          | 1030 w    | ν(Ring1) + ν(Ring2) + ν(Ring3) + ν(Ring4) |
| 1127                          | 1125 w    | ν(C₁₂,₁₃,₁₅,₁₆) + ν(C₁₆-Cl₁)    |
| 1149                          | 1166 m    | ν(Ring1) + ν(Ring2)              |
| 1189                          | 1187 w    | ν(C₉-C₁₀-C₁₁) + δ(C₁₂,₁₅-Hₓ) + δ(C₀-C₁₀-N₅) |
| 1285                          | 1266 w    | ν(Ring1) + ν(Ring4) + δ(C₁₀,₁₃,₁₄-Hₓ) |
| 1415                          | 1411 w    | ν(Ring1) + ν(Ring4) + ν(Ring3) + δ(C₁₅-Hₓ) |
| 1448                          | 1444 w    | ν(Ring1) + ν(Ring2) + ν(Ring3)  |
| 1492                          | 1494 w    | δ(Ring3)                         |
| 1577                          | 1580 w    | ν(N₂-C₉-N₄) + δ(C₁₀,₁₄-Hₓ)       |
| 1613                          | 1606 m    | ν(Ring1)                         |

Note: ν-stretch vibration; δ-bend vibration; s-strong peak; m-medium peak; w-weak peak.

Table 2. The correspondence between the concentrations before and after dilution

| Concentration before dilution (mg/L) | 100 | 75  | 50  | 25  | 10  | 1   |
|---------------------------------------|-----|-----|-----|-----|-----|-----|
| Concentration after dilution (mg/L)  | 40  | 30  | 20  | 10  | 4   | 0.4 |

Note: The calculation formula of concentration before and after dilution as follows:

\[ C_{\text{before}} = (1+1.5) C_{\text{after}} \]  

(1)
Figure Captions

**Schematic 1.** Schematic diagram of the method to detect estazolam by using Au@Ag NPs paper-based substrate.

**Fig. 1** Characterization of nanoparticles; a, SEM image of 46 nm Au NSs; b, SEM image of 60 nm Au@Ag NPs; inset Fig: TEM images of 60 nm Au@Ag NPs; c, 46 nm Au NSs and 60 nm Au@Ag NPs with a statistical graph of the distribution of the nanoparticles; d, UV–Vis spectra of 46 nm Au NSs; inset Fig: macrograph of 46 nm Au NSs and 60 nm Au@Ag NPs.
**Fig. 2** Characterization of substrate; a, Nanoparticles form interfacial film at the oil interface; inset Fig: Macro image of filter paper surface before and after assembly; b, c SEM image of filter paper surface; d, Spatial homogeneity demonstration of the SERS performance using the Au@Ag NPs paper-based substrate; E, The histogram of Raman intensity of MG (1614 cm$^{-1}$).

**Fig. 3** Optimized structure of EZM
**Fig. 4** Raman spectra of the theoretical calculations (black line) and SERS spectrum of the EZM aqueous solution (Red line).

**Fig. 5** The SERS spectra of EZM with concentrations of gradient descent in the aqueous solution; a, The entire spectrum of the EZM concentration gradient; b, The relationship between the Raman signal intensity and sample concentrations at characteristic band of 1000 cm$^{-1}$ and 687 cm$^{-1}$.
**Fig. 6** The stability of this method for detection of EZM; a, SERS spectrum of 15 groups of 50 mg/L EZM solution; b, The relative standard deviation (RSD$_{687} = 5.56\%$ and RSD$_{1000} = 4.35\%$) of the detection.

**Fig. 7** Content determination of EZM in beverage under dilution. The SERS spectra of EZM with concentrations of gradient descent in beverages; a, Orange drink; c, Pomegranate drink.
The relationship between the Raman signal intensity and sample concentrations at characteristic peak of 1000 cm$^{-1}$ and 687 cm$^{-1}$; b, Orange drink; d, Pomegranate drink.

The graphical abstract

Using the Au@Ag SERS paper-based substrate to realize the rapid detection of the psychotropic drug estazolam tablet.

A rapid detection method for on-site screening of estazolam in beverages with Au@Ag core-shell nanoparticles paper-based SERS substrate

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