Aptamer-based fluorescent platform for ultrasensitive adenosine detection utilizing Fe$_3$O$_4$ magnetic nanoparticles and silver nanoparticles

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Abstract

The authors describe an aptamer-based fluorescent assay for adenosine (Ade). It is based on the interaction between silver nanoparticles (AgNPs) and CdTe quantum dots (QDs). The beacon comprises a pair of aptamers, one conjugated to Fe$_3$O$_4$ magnetic nanoparticles, the other to AgNPs. In the presence of Ade, structural folding and sandwich association of the two attachments takes place. After magnetic separation, the associated sandwich structures are exposed to the QDs. The AgNPs in sandwich structures act as the signaling label of Ade by quenching the fluorescence of QDs (at excitation/emission wavelengths of 370/565 nm) via inner filter effect, electron transfer and trapping processes. As a result, the fluorescence of QDs drops with increasing Ade concentration. The assay has a linear response in the 0.1 nM to 30 nM Ade concentration range and a 60 pM limit of detection. The assay only takes 40 min which is the shortest among the aptamer-based methods ever reported. The method was successfully applied to the detection of Ade in spiked biological samples and satisfactory recoveries were obtained.

Keywords

Fluorescence · Ag nanoparticles · Quantum dots · Magnetic separation · Rapid detection · Sandwich structure · Quenching · Urine analysis · Inner filter effect · Lung cancer marker

Introduction

Adenosine (Ade) as an endogenous nucleoside performs extremely important signaling functions in many biological processes such as the anti-arrhythmia, hemangiectasis, increment of the blood flow of the arteries and improvement of the oxygen supply of cardiac muscle [1–3]. Moreover, Ade exhibits an inhibitory effect on synaptic activity and metabolism of the brain, and may also play an important role in the immune system [4]. The normal content of Ade in human body ranges from 0.18 μM to 4.7 μM. Increasing evidences suggest that excessive Ade may indicate tumorigenesis, typically lung cancer [5]. So, direct monitoring of Ade fluctuations under physiological conditions would be of utility in further illustrating their function in cancer clinical diagnosis and treatment.

Several traditional assays, such as the high-performance liquid chromatography [6] and liquid chromatography mass spectrometry [7] allow quantitative analysis of Ade. However, these techniques tend to require expensive instrumentation, sophisticated operation, time-consuming analysis and trained operators. And above all, the foremost limitation of these methods in application of Ade bioassay is insensitivity. In response to these drawbacks, extensive efforts have been contribute to detecting trace levels of Ade by applying an Ade aptamer. Aptamers are single-stranded DNA or RNA (ssDNA or ssRNA) molecules, which are artificially generated from nucleic acids libraries via SELEX, and can bind to pre-selected targets including small organic compounds, macromolecules, viruses and cells with desirable selectivity, specificity and high affinity [8, 9]. In compare with other signal receptors such as antibodies or molecularly imprinted polymers, aptamers show many excellent properties of easy
production, good reproducibility, chemical stability and high affinity [10]. Attributed to their unique three-dimensional folded structures, aptamers can specifically bind to target molecules based on Van der Waals force, hydrogen bonding and hydrophobic function and then undergo significant conformational changes into hairpin, stem-loop, G-quadruplex, pseudoknot or bulge structures [11]. In 1995, a 27-mer aptamer was first reported by Szostak and co-workers, which can specifically recognize and bind to Ade with a transition of its conformation from random coil to hairpin-like [12]. On this basis, extensive efforts focused on developing Ade aptamer-based analytical methods, including colorimetry [13], fluorescence [14], Raman scattering [15], electrochemistry [16–18], chemiluminescence [1], surface plasmon resonance (SPR) [19] and other assays. Among them, fluorescent assays and sensors have attracted considerable attention for their simplicity, speediness, practicability and high sensitivity.

To obtain obvious fluorescent signal, plenty of attempts have been made to decorate fluorescence indicators onto aptamers to develop superior Ade detection platforms. Compared to traditional fluorescein, semiconductor quantum dots (QDs) are new nano inorganic fluorophores that have become attractive tools for bio-labeling and electro-optical applications on account of their unique optical properties, including broad absorption spectra, low photo-bleaching, high quantum yields, good physicochemical stability and size dependent emission wavelength tenability [20]. As a classic and widely investigated semiconductor, CdTe QDs stand out from various tested QDs due to its simplicity of synthetic methods and long-term water solubility [21]. From the perspective of analysis, a large variety of analytical methods have been developed by using CdTe QDs as probes. The modulation of its fluorescence intensity as a specific response to a given target can be noticed either via enhancing or quenching, and the most usual analytical platforms for targets detecting were based, undoubtedly, on the quenching effect [22]. In general, quenching mechanisms are classified into two types: one is based on photo-induced electron transfer (PET) and another is dependent on changes of charge or ligands on the surface of QDs [23]. In the first type, since the electron transfer process between CdTe QDs and some metal ions causes disruption of radiative electron-hole recombination, metal ions have been successfully explored in PET-based sensing schemes by measuring the resulting quenching of CdTe QDs fluorescence [24]. Based on this principle, a signal amplification platform based on the electron transfer reaction between CdTe QDs and Ag⁺ for the monitoring of Ade in the biological fluids had been reported in our previous work [25]. The amplification capability was very effective with the detection limit of 0.217 nM, but the existing problem of this method was the tedious experimental procedure, which came from the twice quantified reactions between Ag⁺ and CdTe QDs and multiple washing and separation operation.

To develop a rapid and handy Ade assay, a more convenient method was proposed by using silver nanoparticles (Ag NPs). Similar to Ag⁺, dramatic quenching of the excitonic emission of CdTe QDs by Ag NPs was based on the first type of quenching mechanisms. Surprisingly, Ag NPs as potential electron reservoirs achieved higher reaction sensitivity than Ag⁺ [26, 27]. To be specific, in the presence of picomole quantities of Ag NPs, a strong fluorescence quenching of CdTe QDs was triggered through inner filter effect and ultrafast electron transfer and charge carrier trapping processes between similarly negatively charged CdTe QDs and Ag NPs [27]. Benefited from this sensitive reaction, a novel fluorescent assay was designed for selective detection of Ade, in which Ade aptamer was employed as biorecognition element and Fe₃O₄ magnetic nanoparticles (MNPs) as magnetic separator. The two split aptamer fragments of Ade aptamer were respectively attached to Fe₃O₄ MNPs and Ag NPs. In the presence of Ade, it would reassemble the two pieces of ssDNA into the intact aptamer tertiary structure to form the stable sandwich structure. After magnetic separation, the amount of Ade in the sandwich structure was quantified by measuring the fluorescence signal of later-added CdTe QDs based on the strong quenching interaction between Ag NPs and CdTe QDs. Moreover, the platform was successfully applied to the determination of Ade in urine samples. Therefore, the platform holds great potential for monitoring various target molecules in the biomedical field and clinical diagnosis.

Experimental section

Materials and reagents

All experiments were performed with analytical reagent grade chemicals and ultrapure water. Glycerol, silver nitrate, sodium chloride, sodium borohydride, tellurium (Te), chromium acetate, sodium hydroxide, absolute ethyl alcohol, 3-mercaptopropionic acid (MPA), ferric chloride and N-hydroxysuccinimide (NHS) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, http://www.sinoreagent.com/). Disodium hydrogen phosphate, sodium dihydrogen phosphate, citric acid monohydrate, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), sodium citrate and ferrous chloride were obtained from Shanghai LingFeng Chemical Co., Ltd. (Shanghai, China, http://lingfenghx.cn.makepolo.com/). Ade, cytidine, uridine and guanosine were all purchased from Shanghai Sangon biological engineering technology & services Co., Ltd. (Shanghai, China, http://www.sangon.com/) which synthesized the oligonucleotides designed according to the literature, and their sequences were shown below: ABA1: 5′-NH₂-C₆-T₆-ACCTGGGGGAGTAT-3′; ABA2: 5′-SH-C₆-T₆-TGCGAGGAAGGT-3′. DNA solutions were prepared
by dissolving originally appropriate DNA in phosphate buffered saline (PBS; saline), then diluting them to the final concentration of 100 μM.

**Apparatus**

UV-visible absorption spectra were measured with a Shimadzu UV2450 spectrometer (Shimadzu, Japan, https://www.shimadzu.com.cn/). FT-IR spectra were recorded on a TENSOR 27 spectrometer (Bruker, Germany, https://www.bruker.com/) with a resolution of 2 cm⁻¹ and a spectral range of 4000–400 cm⁻¹. All fluorescence measurements were completed on a Hitachi F-4600 spectrofluorometer (Hitachi, Japan, http://www.hitachi.com/). The transmission electron microscopic (TEM) image of Ag NPs was performed on a Phillips FEI Tecnai G2 Spirit Bio TWIN (FEI Company, USA, https://www.fei.com/tecnai-upgrades/). The TEM image of CdTe QDs was acquired on a Tecnai G2 F30 S-TWIN (FEI Company, USA, https://www.fei.com/tecnai-upgrades/). The magnetic properties were studied by vibrating sample magnetometer (VSM, PPMS Dynacool, https://www.uni-due.de/physik/wende/ppms_en.php) at room temperature. The electrochemical measurements were performed in a conventional three-electrode cell, using a CHI 760C workstation (Shanghai Chenhua Co., China, http://www.chinstr.com/) with a platinum electrode as the reference electrode and a glassy carbon electrode (GCE, Tianjin Incole Union Technology Co., China, http://www.tianjinincole.com/) as the working electrode.

**Preparation of the ABA1-modified Fe₃O₄ magnetic nanoparticles (MNPs)**

The synthetic procedure of carboxyl-modified Fe₃O₄ MNPs (Fe₃O₄-COOH) is shown in Electronic Supporting Material. 0.01 g of comminuted Fe₃O₄-COOH was ultrasonic dissolved in 1 mL of phosphate buffered saline (pH 5.7), then 500 μL of 0.05 M EDC/NHS solution was added to the suspension and the mixture was stirred at room temperature for 30 min. The activated Fe₃O₄-COOH was magnetic separated and washed for three times and then dissolved in 1 mL of phosphate buffered saline (pH 7.4). Then 20 μL of 100 μM ABA1 was added to the activated suspension and the mixture was incubated at 25 °C for 12 h to facilitate the reaction. Afterwards, Fe₃O₄-COOH bound with ABA1 was washed with saline buffer (0.01 M, pH 7.4) for three times. Finally, the ABA1-modified Fe₃O₄ MNPs were dispersed in 100 μL of buffered saline (0.01 M, pH 7.4) and stored at 4 °C.

**Synthesis of ABA2-modified Ag NPs**

The synthetic procedure of Ag NPs is shown in Electronic Supporting Material. To synthesize the ABA2-modified Ag NPs, 1 mL of Ag NPs solution with 10 μL of 100 μM ABA2 was incubated for at least 18 h. Then 122 μL of PBS (0.01 M, pH 7.4) was added to the solution to adjust the pH value and increase ionic strength of the resulting solution. 6 h later, 21 μL of 2 M NaCl was added to the solution and this procedure was repeated two times at the interval of 3 h such that the total NaCl concentration was gradually increased. After an additional standing for at least 48 h, the nanoparticles were isolated by centrifugation at 15000 rpm for 15 min.

**Detection of Ade**

Different concentrations of Ade (0.1, 0.2, 0.5, 1, 5, 10, 15, 20, 25 and 30 nM) were prepared with an Ade stock solution (1 μM) in PBS (0.01 M, pH 7.4). 100 μL of each Ade standard solution was added to the mixture of 100 μL of ABA1-modified Fe₃O₄ MNPs and 100 μL of ABA2-modified Ag NPs, and the mixture was incubated at room temperature for 30 min to form sandwich structures. Afterwards, the sandwich structures were magnetically separated and washed with PBS (0.01 M, pH 7.4) for three times, and then re-dispersed in 100 μL PBS (0.01 M, pH 7.4). Then, 1 mL of 2 mM CdTe QDs solution was added to the sandwich structure. When the mixture solution was evenly blended, the fluorescence intensities of mixture solution in presence of Ade (denoted as F) and absence of Ade (denoted as F₀) were measured.

**Preparation of urine samples**

With the informed-consent, freshly urine samples were obtained from three health volunteers. The urine specimens were filtered through a 0.2 μm membrane, and 100 μL of the filtrate was diluted to 5 mL with PBS (pH 7.4). The amount of Ade in the diluted sample solution was detected as described in Detection of Ade.

**Results and discussion**

**Strategy of the Ade assay**

A novel Ade fluorescent assay was successfully constructed by uniting Ade aptamer as recognition unit and Fe₃O₄ MNPs as magnetic separator. Firstly, Ade aptamer was cut into two pieces of ssDNA (ABA1 and ABA2) shown in Fig. S1, while two Ade molecules reassembled a unit of ABA1 and ABA2 into the intact aptamer tertiary structure within 30 min [28]. As described in Scheme 1, in order to achieve rapid separation of the target Ade from the complicated biological samples, the terminal amino-modified ABA1 was decorated with the Fe₃O₄-COOH via covalent linkage, while the terminal thiol-modified ABA2 was attached to Ag NPs through Ag-
S bond to obtain the signal label, and these two conjugates were mixed. With the advent of samples in the second step, a unit of the ABA1-modified Fe$_3$O$_4$ MNPs and ABA2-modified Ag NPs self-assembled to form sandwich structure by capturing two Ade molecules specifically with the incubation for 30 min. Conversely, the two attachments did not bind to form the sandwich structure in absence of Ade. Once the sandwich structure and redundant ABA1-modified Fe$_3$O$_4$ MNPs were isolated from the mixture and re-dispersed in buffer through magnetic separation and wash processes in the third step, a certain amount of CdTe QDs was added. Finally, the fluorescence intensity of the mixture solution was measured. Ag NPs in sandwich structure, which amount was in proportion to the amount of Ade in samples, exhibit strong fluorescence quenching function on CdTe QDs, leading to the final fluorescence signal output gradually decreased with the increasing Ade concentrations. In this design, there exists not only the shorter detection duration and the easier operating steps, but great improvement of the sensitivity to detect Ade which benefited from the ultrasensitive reaction between CdTe QDs and Ag NPs.

Figure 1a shows that the maximum excitation wavelength and the maximum emission wavelength of CdTe QDs are 370 nm and 565 nm respectively (curve a, b); while the distinct surface plasmon band of Ag NPs is at 405 nm (curve c). Apparently, the excitation spectra of CdTe QDs overlaps the absorption spectra of Ag NPs, leading to the fluorescence quenching of the former close to the latter surface due to the inner filter effect [29]. Moreover, Ag NPs as potential electron reservoirs promote ultrafast electron transfer and charge carrier trapping processes between Ag NPs and CdTe QDs, and then exhibit strong fluorescence quenching effect on CdTe QDs [27]. As can be seen in Fig. 1b, Ag NPs at pM level of concentrations (3 to 300 pM) quench the fluorescence of CdTe QDs ($\lambda_{\text{ex/em}} = 370/565$ nm), and the quenching intensity in this system followed equation: $F/F_0 = 0.9806e^{-0.005C}$ with a correlation coefficient of 0.99 (where, F and $F_0$ are the fluorescence intensity of CdTe QDs in the presence and absence of Ag NPs respectively, C is the concentration of Ag NPs). The non-linear trend (120 to 300 pM) indicates the quenching progresses is combined quenching, where both static and dynamic quenching are present in the system. In fact, static quenching happens with the formation of non-fluorescent complex, whereas dynamic quenching is due to collisions between fluorophore and the quencher [30]. In this case, the static quenching may arise from the inner filter effect, while the electron transfer and charge carrier trapping processes trigger the dynamic quenching. As a result, this intense reaction provides strong support for the ultrasensitive detection of trace amount of Ade in biological samples.

Scheme 1  Schematic illustration of the Ade detecting procedure
Characterization of the ABA1-modified Fe$_3$O$_4$ magnetic nanoparticles (MNPs)

To confirm the carboxyl groups were modified on the surface of the Fe$_3$O$_4$ MNPs, FT-IR spectra of Fe$_3$O$_4$ MNPs and Fe$_3$O$_4$-COOH are shown in Fig. S2 (curve a, b) respectively. The obvious peaks at 590 cm$^{-1}$ corresponds to the stretching vibration of Fe-O bond. The peaks at 1408 cm$^{-1}$ and 1625 cm$^{-1}$ can be attributed to the stretching vibrations of C-O bond and C=O bond, respectively, while the peak at 3418 cm$^{-1}$ corresponds to the stretching vibration of dissociative hydroxyl groups, which suggests the successful coating of carboxyl group on the surface of Fe$_3$O$_4$ MNPs [31]. As demonstrated in Fig. S3, the Fe$_3$O$_4$-COOH not only has a good dispersion in aqueous solution, but exhibits a strong magnetism, in which the Fe$_3$O$_4$-COOH quickly separated to the side of the sample vial close to magnet within 10 s. The UV-visible absorption spectra of ABA1, Fe$_3$O$_4$-COOH and Fe$_3$O$_4$-ABA1 are shown in Fig. 2a (curves a, b and c). It can be seen that ABA1 has a characteristic absorption peak at 258 nm and Fe$_3$O$_4$-COOH has wide absorption, while the ABA1-modified Fe$_3$O$_4$ MNPs both have wide absorption and a characteristic absorption peak at 258 nm attributed to ABA1. According to the synthetic process of ABA1-modified Fe$_3$O$_4$ MNPs, the unlinked ABA1 were removed through water washing and magnetic separation steps. Therefore, the characteristic UV-visible absorption signal of ABA1-modified Fe$_3$O$_4$ MNPs is totally ascribed to ABA1 that linked to the surface of Fe$_3$O$_4$ MNPs, reflecting ABA1 is well conjugated with Fe$_3$O$_4$-COOH. Additionally, Fig. 2b shows that the magnetic hysteresis loops of the Fe$_3$O$_4$-COOH and Fe$_3$O$_4$:ABA1 exhibit super paramagnetic behavior with the magnetic saturation value are 63.43 emu g$^{-1}$ and 58.20 emu g$^{-1}$ respectively (curves a, b), indicating that the modification of ABA1 on the surface of Fe$_3$O$_4$ MNPs has no influence on the magnetism of Fe$_3$O$_4$ MNPs, which become efficient separator from complicated matrices by applying a magnetic field [32].

Characterization of the ABA2-modified silver nanoparticles (Ag NPs)

Different techniques were utilized to verify that the Ag NPs were decorated with terminal thiol-modified ABA2 through Ag-S bond. Figure 3a represents a TEM image of the citrate-capped Ag NPs. The regularly spherical particles exhibit a good dispersity and size homogeneity with the granular diameter of 30 nm, contributing to the well decoration of ABA2 on their surface. The UV-visible absorption spectra of ABA2, Ag NPs and Ag NPs-ABA2 are shown in Fig. 3b (curves a, b and c). It can be seen that the ABA2 and Ag NPs have a characteristic absorption peak at 258 nm and 405 nm respectively, while the compound Ag NPs-ABA2 has the same wavelength absorbance maximum as the Ag NPs at 405 nm and a broader absorbance peak at 200-300 nm. Since the unlinked ABA2 was removed through centrifugation, the distinction between absorption of ABA2-modified Ag NPs and bare Ag NPs is totally ascribed to ABA2 that linked to the surface of Ag NPs. In addition, the measured Zeta potentials shown in Fig. S4 (-16.7 mV and -26.7 mV for Ag NPs and Ag NPs-ABA2.
respectively) confirm that the negative charged ABA2 linked to Ag NPs [33].

Cyclic voltammograms (CVs) were acquired to evaluate the electrochemical sensing performance of ABA2 before and after reacting with Ag NPs. As depicted in Fig. 3c, compared with bare GCE (curve a), ABA2/GCE exhibits smaller peak current with larger peak potential separation (curve b), which is ascribed to the weaker electrical conductivity of ABA2. While ABA2 is decorated with Ag NPs, the peak current is about 1.6 times larger and the peak potential separation (88 mV, curve c) is much smaller than that of bare ABA2, which is attribute to the excellent electrical conductivity of Ag NPs. Accordingly, electrochemical impedance spectroscopy (EIS) is used to investigate the electron transfer resistance features of ABA2 before and after linked to Ag NPs by using $[\text{Fe(CN)}_6]^{3-/4-}$ as redox probe. The semicircle diameter of EIS is represented as the electron transfer resistance ($R_{et}$). As illustrated in Fig. 3d, compared to $R_{et}$ of bare GCE (1692 Ω, curve a), the semicircle diameter of ABA2/GCE increases dramatically to 47,435 Ω (curve b), which is due to the much weaker conductivity of ABA2 than that of bare GCE. As expected, after linked to Ag NPs, the semicircle diameter of Ag NPs-ABA2/GCE ($R_{et} = 1439$ Ω, curves c) decreases significantly compared to that of ABA2/GCE, and this sharply decrease of $R_{et}$ is ascribed to the Ag NPs which greatly promote the electron transfer. The EIS behaviors of ABA2 before and after reacting with Ag NPs are consistent with CV performances. The results of CV and EIS of ABA2 before and after reacting with Ag NPs indicate that ABA2-modified Ag NPs are successfully prepared.

### Optimization of experimental conditions

The following parameters were optimized: (a) Fe$_3$O$_4$ MNPs concentration; (b) ABA1 volume. Respective experimental conditions are as follows:

- **Fe$_3$O$_4$ MNPs concentration**: The concentration of Fe$_3$O$_4$ MNPs was varied from 0.1 to 1 mg/mL.
- **ABA1 volume**: The volume of ABA1 solution was varied from 0.1 to 1 mL.

The optimal conditions were determined to be 0.5 mg/mL Fe$_3$O$_4$ MNPs and 0.5 mL ABA1 solution.

### Selective performance of the analytical method

The selective performance of the analytical method to Ade and interferent: three Ade analogs (a); other common interferent (b, from b to t: Na$^+$, K$^+$, Ca$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Cu$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Pb$^{2+}$, H$_3$PO$_4^-$, HPO$_4^{2-}$, Cl$^-$, ClO$^-$, SO$_4^{2-}$, HCO$_3^-$, Glu, Urea and UA). The concentration of Ade and each interferent were 10 nM and 1 μM, respectively.
data and Figures are given in the Electronic Supporting Material. The following experimental conditions were found to give best results: (a) Fe$_3$O$_4$ MNPs concentration: 0.01 g mL$^{-1}$; (b) ABA1 volume: 20 μL.

Selectivity and interference

The Ade analogs uridine, cytidine and guanosine were evaluated at a concentration of 1 μM, which was 100 times that of Ade (10 nM). As shown in Fig. 4a, only Ade at 10 nM gives a significant fluorescence quenching while the three Ade analogs nearly do not cause the decrease of the fluorescence intensity of CdTe QDs in comparison with the background signal, revealing that this aptamer-based assay has a superior selectivity for Ade. In addition, to clarify that the biological matrix do not cause interference, metal ions, anions, glucose (Glu), urea and urea acid (UA) were investigated at a concentration of 1 μM while the concentration of Ade was 10 nM, and no obvious interfering effects are observed as shown in Fig. 4b. Considering this issue, the selectivity of the system was satisfactory.

Validation of the method

Under the optimum conditions, the fluorescence intensity of the system ($\lambda_{ex/cm} = 370/565$ nm) linearly decreases with the increment of Ade in the range of 0.1 nM – 30 nM as demonstrated in Fig. 5. The linear equation is $F/F_0 = 0.9837 - 0.0301C$ with a coefficient of association of 0.99 where, $F$ and $F_0$ are the fluorescence intensity of Ade detection system in the presence and absence of Ade, respectively, $C$ is the concentration of Ade. The limit of detection of 0.06 nM is obtained based on 3σ/k (where, σ is the standard deviation of blank measurements ($n = 10$), and k is the slope of calibration graph).

The comparison between this method and other aptamer-based Ade detection techniques is summed up in Table 1. The platform possesses several remarkable features compared to previous Ade assays: (1) As the signaling label of Ade, Ag NPs exhibit highly sensitive and very fast quenching reaction to CdTe QDs, which guarantees the extremely sensitivity and satisfying analytical performance for sensing. (2) Specifically, in comparison to Ade aptasensors ever reported, the platform as the fastest level holds great potential for highly efficient monitoring Ade concentration in the biomedical field and clinical diagnosis. Moreover, we used the intra-day and the inter-day assay to investigate the accuracy and precision by analyzing three samples with low (0.2 nM), medium (5 nM), and high (25 nM) levels of Ade concentration. As shown in Table S1 in supporting information, the relative standard deviations (RSDs) ranging from 0.22% to 5.26% indicate that the assay has acceptable levels of accuracy and precision.

Analysis of real samples and evaluation of method accuracy

To evaluate the practicability of the method, the concentration of Ade in human urine samples from three healthy volunteers were detected. The experiment results were summarized in Table S2 in supporting information, and the recovery values

![Fluorescence spectra of the assay ($\lambda_{ex/cm} = 370/565$ nm) in the presence of different concentrations of Ade (from a to k: 0, 0.1, 0.2, 0.5, 1, 5, 10, 15, 20, 25, 30 nM). Inset: Calibration plot: fluorescence intensity change $[F/F_0]$ versus Ade concentration (0.1 nM–30 nM).]
ranged from 91.2% to 106%. As depicted in Fig. 6, there was no significant difference between this method and HPLC (the chromatography conditions were given in Electronic Supporting Material), demonstrating that the method has a large potential in clinical detection of Ade without any sample pretreatments.

**Conclusion**

To summarize, a novel and convenient Ade platform based on the ultrasensitive reaction between Ag NPs and CdTe QDs was fabricated, which united Ade aptamer as recognition unit and Fe₃O₄ MNPs as magnetic separator. The results indicated that the high selectivity for Ade and excellent sensitivity with a wide detection range of 0.1 nM – 30 nM and a detection limit of 60 pM. Furthermore, the platform holds great potential for monitoring various target molecules in the biomedical field and clinical diagnosis for its high efficiency and low cost. We will focus on exploring this method for the development of diagnostic kit in the future.

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