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Original Article

Gold nanoparticles grafted modified silica gel as a new stationary phase for separation and determination of steroid hormones by thin layer chromatography

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ABSTRACT

A new thin layer chromatographic layer using gold nanoparticles grafted 3-triethoxysilyl propylamine modified silica gel (Au NPs-APTS modified silica gel) was developed as a stationary phase for separation and determination of two steroid hormones, namely progesterone and testosterone. Acetone–n-hexane 25:75 (v/v) was used as the mobile phase, and the results were compared with those obtained using plain (i.e., unmodified) silica gel plates. Some chromatographic parameters used for separation of the two steroids on an Au NPs-APTS modified silica gel plate as well as on a plain silica gel plate, including ΔR_F , separation factor (α), and resolution (R_S), were evaluated and compared. The reproducibility of R_F values was also determined by analysis of the two steroids in 7 consecutive days on both plates. Validity of the method was investigated, and a wide linear range of 1–200 ng per spot, and low detection limits of 0.16 ng and 0.13 ng per spot, low quantification limits of 0.51 ng and 0.40 ng per spot, and good precision (expressed as percent relative standard deviation) lower than 3.1% and 2.7% were obtained for progesterone and testosterone, respectively. As the results revealed, the proposed method is rapid and sensitive, and it is applicable to separation and determination of progesterone and testosterone in biological matrices such as urine samples.

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1. Introduction

Steroid hormones are widely used to improve feed conversion efficiency and growth rates [1]. Many governmental regulatory agencies have dedicated much effort to develop analytical

methods for monitoring hormones. However, assessment of natural steroids has been an analytical challenge due to their extremely low concentration and presence of interferences in biological matrices [2].

Recently, the role of planar chromatography, especially thin layer chromatography (TLC), has systematically increased [3].

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The main advantages of TLC over other specific liquid–mobile phase separation techniques, including high-performance liquid chromatography and capillary electrophoresis (CE), are the use of inexpensive equipment, lower purity of consuming solvents, saving a significant amount of consumables, and better waste removal [4]. Although unmodified silica gel or C_{18} functionalized silica are typically used as stationary phases for many TLC methods, some drawbacks impede their use in certain applications, such as peak tailing and dewetting in highly aqueous environment [5]. During the past few decades, many efforts have been made focusing on the improvement of the chromatographic properties of silica-based stationary phases. Furthermore, several new stationary phases have been developed, providing users alternative selectivity for more difficult chromatographic separations [6–10].

Recently, nanotechnology has led to innovations in various fields of separation science. Specially, nanostructured materials with a large surface-to-volume ratio and specific chemical properties attracted more and more attention for their potential application in chromatographic separations [11]. For example, the use of gold nanoparticles (Au NPs) has gradually been increased due to their ease of preparation, controllable particle size, narrow size distribution, good solubility, and convenient modification [11]. Significant advances have been achieved using Au NPs in gas chromatography, TLC, and CE methods. Gross et al [12] reported the first application of Au NPs in gas chromatography. They prepared monolayer-protected Au NPs by covalent immobilization of dodecanethiol on gold surface. The gold modified stationary phase provided fast mass transfer, and it can be used for specific applications based on the known gold chemistry. Shapovalova et al [13] reported the first use of these NPs for immobilization of L-cysteine on TLC plates. The first article describing the application of Au NPs in CE was published by Neiman et al [14] in 2001. Two different Au NPs (citrate and thiol stabilized) were used to generate a pseudostationary phase. The authors ascertained that although both modified Au NPs provided efficient and repeatable CE separations, the thiol-stabilized Au NPs resulted in better CE systems. In 2006 Yu et al [15] utilized surfactant modified Au NPs for CE separation of acidic and basic proteins. Although all the above-mentioned methods are sensitive, they are rather complex and have complex, tedious, and very time-consuming sorbent preparation steps, along with laborious column packing and pretreatments. Recently, three steroids (progesterone, testosterone, and testosterone propionate) were successfully separated on CE columns prepared through the alkanethiol self-assembly and dithiol layer-by-layer self-assembly processes onto Au NPs by Liu [16,17]. It was found that silica gel, which is coated by Au NPs self-assembled layer by layer with alkanethiols, is a good solid-phase extraction material for CE determination of natural steroids in urine samples. In fact, unreacted Au NP surface can eliminate urinary proteins. Thus, we suspected that the use of Au NPs in the structure of silica stationary phase could minimize baseline interferences of biological fluids and improve the sensitivity of TLC determination. Therefore, the suggested method enables quick, efficient, and sensitive measurements of the steroids with minimal baseline interferences.

This study mainly focused on the development of a simple, cost-effective, and sensitive TLC method based on the use of

Au NPs grafted 3-triethoxysilyl propylamine (APTS) modified silica gel (Au NPs-APTS modified silica gel) as an efficient stationary phase to separate steroid hormones. Some chromatographic parameters such as ΔR_f , separation factor (α), and resolution (R_s) for separation of the two steroids were evaluated, and the method was applied for the separation and determination of progesterone and testosterone (Fig. 1) from urine samples. Although, Au NPs were used previously to modify conventional stationary phases, to the best of our knowledge, this is the first report dealing with the use of a new Au NP-based modified silica gel as a stationary phase in TLC for the separation of the steroids.

2. Materials and methods

2.1. Materials and reagents

All chemicals and reagents were of analytical grade and used without further purification. Hydrogen tetrachloroaurate ($H AuCl_4$), silica gel, zinc sulfide, calcium sulfate, APTS, trisodium citrate, nitric acid, hydrochloric acid, methanol, ethanol, acetone, and n-hexane were purchased from Merck Company (Darmstadt, Germany). Progesterone and testosterone standards were obtained from the Center of Quality Control of Drug, Tehran, Iran, and deionized water was used throughout the experiments.

Stock solutions of progesterone and testosterone (1000 $\mu g/mL$) were prepared by dissolving 100 mg of each analyte in 100 mL methanol. The solutions were stored at 4°C in dark and were stable for at least 2 weeks. The working standard solutions were prepared in the concentration range of 1–200 $\mu g/mL$ for construction of a calibration curve, and all dilutions were made with methanol.

2.2. Preparation of colloidal Au NPs

Colloidal Au NPs were prepared by citrate reduction of gold ions in water following the method introduced by Turkevich et al [18] in 1951, with slight modifications. Briefly, a solution of 5mM $H AuCl_4$ (2 mL) was added to deionized water (45 mL) in a conical flask and allowed to boil with constant stirring under reflux. The reaction was followed by the addition of 25mM of trisodium citrate (3 mL) under continuous stirring. The color of

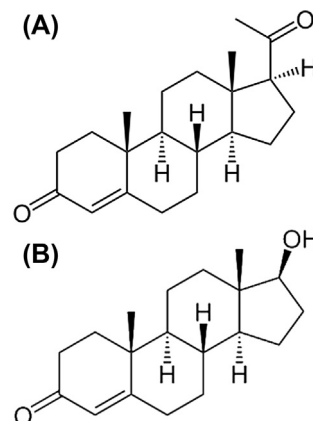


Fig. 1 – Structure of (A) progesterone and (B) testosterone.

the solution changed from almost colorless to typical deep red. The reaction was kept in the same condition for 5 minutes, and then it was rapidly cooled to room temperature using an ice bath. UV–Vis spectroscopy (model UV-240, UV–Vis spectrophotometer; Shimadzu, Kyoto, Japan) and transmission electron microscopy (model CM30; Philips, Amsterdam, The Netherlands) were used to determine the mean diameter of Au NPs, and their average size was found to be 15 ± 5 nm (Fig. 2). Zeta potential (Zetasizer; Malvern Instruments Ltd, Malvern, UK) measurements revealed that the NPs obtained had an average surface charge of -43.3 ± 0.9 mV.

2.3. Preparation of stationary phases

The method used for coating of Au NPs onto the modified silica gel was similar to that described previously [17], with

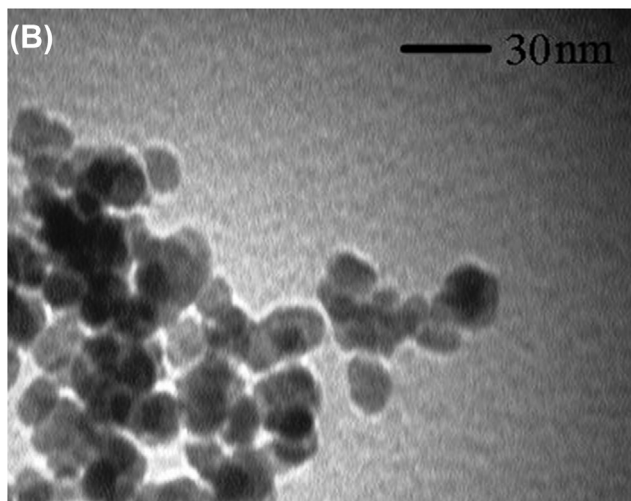
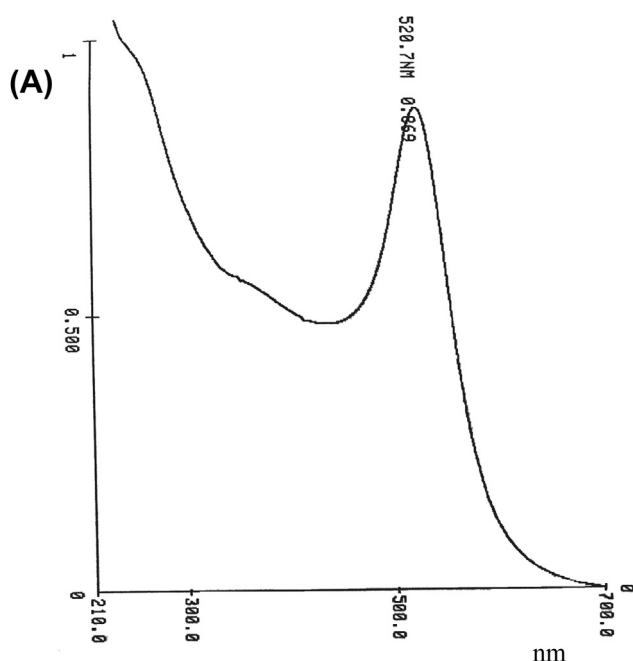


Fig. 2 – (A) UV–Vis spectrum and (B) TEM image of the prepared Au NPs. Au NP = gold nanoparticle; TEM = transmission electron microscopy; UV–Vis = ultraviolet–visible.

slight modifications; the schematic illustration of chemical grafting procedure is presented in Fig. 3. Briefly, silica gel (10 g) was added to concentrate HCl (20 mL) in a 100 mL round bottom flask, heated under reflux for 2 hours, and then washed with deionized water to neutralize. After centrifugation (1650 g, 10 min), the activated silica gel (as plain silica gel) was placed in an oven and dried at 100°C for 24 hours to remove all moisture. Then, the silica powder was added to a solution of 1% (v/v) APTS in ethanol (100 mL), stirred vigorously for 2 hours and left to stand overnight. Finally, the suspension was decanted and annealed at 100°C in an oven for 24 hours to prepare APTS modified silica gel. In the next step, a solution of Au NPs (100 mL, 0.2mM) was mixed with the APTS modified silica gel and stirred for 2 hours. The suspension was allowed to settle, and excess Au NPs were removed by several washing/decantation steps with deionized water. The prepared Au NPs-APTS modified silica gel was placed in an oven and dried at 50°C for 24 hours. In another experiment, activated or plain silica gel was mixed with the colloidal Au NPs (100 mL, 0.2mM) with vigorous stirring for 2 hours. After washing/decantation procedure as mentioned for Au NPs-APTS modified silica gel. Finally, the prepared Au NPs modified silica gel was dried at 50°C in an oven.

2.4. Preparation of TLC plates

TLC plates were prepared on 10 cm \times 20 cm clean glass plates. Slurry of each stationary phase material, including Au NPs-APTS grafted, Au NPs modified, APTS modified, and plain silica gel (10 g), with ZnS (1.5 g) as a fluorescence agent and calcium sulfate (3.5 g) as a binder, in 100 mL deionized water was placed on glass plates and spread uniformly to form a 300 μm layer. The plates were left to air dry overnight on the laboratory bench top and were ready to use the following day. The prepared plates were white except for the one containing Au NPs-APTS modified silica gel plate, which was pale pink and used without further pretreatments.

2.5. Chromatographic procedure

The chromatographic procedure was performed on the four plates prepared in this study. Standard solutions of the steroids (1.0 μL) were spotted at about 20 mm above the lower edge of the plates, 10 mm from the side edges, and 7.5 mm apart using a Nanomat 4 sample applicator (CAMAG, Muttenz, Switzerland) at an application rate of 0.1 $\mu\text{L}/\text{second}$. The spots were allowed to dry, and then the plates were developed in a chromatographic chamber (length 27 cm \times width 7.5 cm \times height 25 cm) at room temperature ($25 \pm 2^\circ\text{C}$), pre-saturated with the desired solvent system for 20 minutes. Different mobile phases, including n-hexane–acetone, n-hexane–acetone–acetic acid, n-hexane–methanol, and n-hexane–methanol–acetic acid, with different compositions were evaluated for the separation. Finally, the steroids were developed using a mobile phase of n-hexane–acetone 75:25 (v/v), and the solvent ascend was kept up to 10 cm from the point of application with the development time of about 40 minutes. The volume of mobile phase used per development stage was 20 mL. After development, the plates were left to dry at an ambient temperature in air.

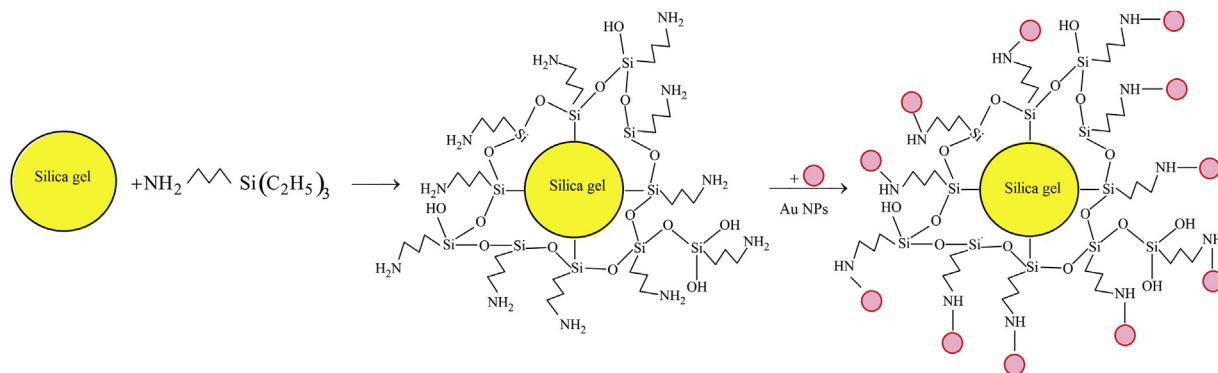


Fig. 3 – Schematic illustration of preparation of Au NPs-APTS modified silica gel stationary phase. Au NPs-APTS = gold nanoparticles grafted 3-triethoxysilyl propylamine.

2.6. Visualization and determination of the steroids

The steroid hormones were visualized by exposing the plates to iodine vapor. About 1 g of solid iodine was transferred to a 100 mL beaker and placed in a water bath to vaporize. The plates were kept above the beaker so that the spots were exposed to the iodine vapor. Individual standards were used alongside the steroids mixture, and after development, the R_F values were calculated from the values of R_L (R_F of the leading front) and R_T (R_F of the tailing front) using the following equation [19]:

$$R_F = 0.5(R_L + R_T) \quad (1)$$

Densitometric scanning was performed at 254 nm using a CAMAG TLC scanner 3 with Cats 4 software (Muttentz) in absorbance mode. This wavelength was selected after acquiring the UV spectra of the analytes, and peak areas were used for quantitative analysis. The slit dimensions were 5 mm × 0.45 mm, and the scanning speed was 10 mm/s. The radiation source was a deuterium lamp emitting continuous UV radiation between 190 nm and 360 nm.

2.7. Chromatographic parameters

Stability of the steroid standards and reproducibility of the R_F values were examined. For this purpose, equal volumes of the steroid standard solutions were mixed and 1.0 μ L of the resultant mixture was loaded on Au NPs-APTS modified and plain silica gel plates. The plates were developed with the mobile phase and dried at an ambient temperature in air, and the spots were detected by iodine vapor. The same process was repeated at intervals of 24 hours for 7 days.

The chromatographic parameters such as ΔR_F , resolution (R_S), and separation factor (α) were determined by the following equations [20,21]:

$$\Delta R_F = R_{F(\text{PRO})} - R_{F(\text{TES})} \text{ [where } R_{F(\text{PRO})} > R_{F(\text{TES})}] \quad (2)$$

$$K_{\text{PRO}} = (1 - R_{F(\text{PRO})}) / R_{F(\text{PRO})} \quad (3)$$

$$K_{\text{TES}} = (1 - R_{F(\text{TES})}) / R_{F(\text{TES})} \quad (4)$$

$$\alpha = K_{\text{PRO}} / K_{\text{TES}} \quad (5)$$

where $R_{F(\text{PRO})}$ and $R_{F(\text{TES})}$ are the R_F values of progesterone and testosterone, respectively. Resolution was calculated using the chromatographic data obtained from the visual and densitometric methods. $R_{S,V}$ values obtained by the use of the visual method were calculated using the following equation [20,22,23]:

$$R_{S,V} = 2\Delta R_F / (D_{\text{PRO}} + D_{\text{TES}}) \quad (6)$$

where D_{PRO} and D_{TES} are the diameters of the two spots. Peak resolutions $R_{S,b}$ [14–16] and $R_{S,h}$ [14,15] were calculated using Equations (7) and (8), and the average peak resolution ($R_{S,D}$) was calculated using Equation (9):

$$R_{S,b} = 2\Delta R_F / (W_{b,\text{PRO}} + W_{b,\text{TES}}) \quad (7)$$

$$R_{S,h} = \sqrt{\ln 4} \times \Delta R_F / (W_{h,\text{PRO}} + W_{h,\text{TES}}) \quad (8)$$

$$R_{S,D} = (R_{S,b} + R_{S,h}) / 2 \quad (9)$$

where $W_{b,\text{PRO}}$ and $W_{b,\text{TES}}$ are the peak widths at baseline, and $W_{h,\text{PRO}}$ and $W_{h,\text{TES}}$ are the peak widths at half height for progesterone and testosterone, respectively.

2.8. Sample preparation

Urine samples were collected in large containers without adding preservatives from seven male healthy volunteers aged between 24 years and 32 years who had given their written consent. All samples were stored at -16°C in a freezer until use. Prior to analysis, the samples were left to thaw and equilibrate to the room temperature. Each sample was centrifuged at 1650 g for 15 minutes, and 1.0 μ L of the clear supernatant was injected into the TLC plate. For recovery tests, the supernatants were spiked by appropriate amounts of the steroids and subjected to the same analysis as the blank samples.

2.9. Validation of the method

The method was validated in terms of specificity, linearity, limit of detection, limit of quantification, precision, and accuracy [24]. The specificity of the method was determined by comparing chromatograms obtained from standard solutions

containing the active substances with those obtained from sample solutions, and peak identities were confirmed by comparison of R_F values.

Linearity of the method was determined by five replicate applications of six standard solutions in the concentration range of 1–200 $\mu\text{g/mL}$, and six-point calibration plots were constructed in the range of 1–200 ng per spot (1 ng, 25 ng, 50 ng, 100 ng, 150 ng, and 200 ng per spot) by plotting the peak area against the corresponding amount of the steroids, using the least squares method. The limit of detection and limit of quantification were determined on the basis of the standard deviation of intercept (S_b) and slope (m) of the straight lines obtained from the regression equations as follows:

$$\text{Limit of detection} = 3.3S_b/m \quad (10)$$

$$\text{Limit of quantification} = 10S_b/m \quad (11)$$

The precision of the method was estimated as repeatability or intraday precision and intermediate or interday precision by intra- and interday analysis of the steroids at three different levels (5 ng, 50 ng, and 150 ng per spot) in five replicates on three different plates, and the results were expressed as percent relative standard deviation (RSD%). The accuracy of the method was evaluated by determination of recovery at three different levels, after the addition of 10 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, and 50 $\mu\text{g/mL}$ of standard solutions (corresponding to 10 ng, 25 ng, and 50 ng per spot) to the urine samples.

3. Results and discussion

The aim of this work is the development of a simple, rapid, sensitive, and reliable analytical procedure for determination of steroid hormones, in order to eliminate the demand for a complicated or/and expensive instrument such as immunoassay, high-performance liquid chromatography–tandem mass spectrometry, and gas chromatography–tandem mass spectrometry. For this purpose, TLC separation of progesterone and testosterone was tried using the four TLC plates prepared in this study. Plain (unmodified), APTS modified, Au NPs modified, and Au NPs-APTS modified silica gel layers were examined. The results revealed that Au NPs cannot attach to the unmodified silica gel completely. Therefore, the R_F values obtained using the four TLC plates were not different from those obtained using plain silica gel. By contrast, APTS modified silica gel was a very polar stationary phase and the hormone spots were not separated on this plate. Thus, further studies were performed and compared using the plain and Au NPs-APTS modified silica gel plates, and the results revealed that the obtained R_F values were 0.65 and 0.89 on Au NPs-APTS modified silica gel and 0.88 and 0.96 on plain silica gel plates for progesterone and testosterone, respectively. As can be seen, the R_F value of progesterone was more influenced by the Au NPs-APTS modified silica gel plate. In fact, silica gel is a polar stationary phase and cannot interact effectively with the nonpolar steroids, as revealed by the obtained R_F values. Interaction with Au NPs can decrease the polarity of silica gel. Thus, progesterone and testosterone interact more with the layer. Progesterone and testosterone have log p values of 3.58

and 2.99, respectively (<http://www.vcllab.org/lab/alogps>). Since testosterone is slightly more polar than progesterone (low hydrophilicity and therefore a high log p value), it was less influenced by the layer, leading to a higher R_F value. Thus, compared to the plain silica layer, better separation of the steroids was achieved.

3.1. Chromatographic parameters

Chromatographic parameters such as ΔR_F , R_S , and α were calculated for progesterone and testosterone on both Au NPs-APTS modified and plain silica gel plates, and the results are presented in Table 1. From the table, it is clear that better separation was obtained on Au NPs-APTS modified silica gel, as indicated by higher values of ΔR_F , R_S , and α . The minimum ΔR_F value indicative of good separation was accepted as 0.04 [20,25]. Both densitometric and visual methods were used for the calculation of peak resolution. Visual evaluation may be subjective and not very precise compared with the densitometric method. Hence, as can be seen from the table, the R_S values obtained by use of the visual method were much higher than those obtained by densitometry [20,26]. Densitometry provides an objective evaluation of the separation effect and the characteristics of particular chromatographic bands. For complete separation of neighboring compounds on a densitogram, R_S must be > 1.5 [20,27]. The obtained results indicate that better resolution (R_S values > 1.5) was obtained for the two steroids on Au NPs-APTS modified silica gel plates. Reproducibility of R_F values for the resolved spots was also determined on the Au NPs-APTS modified silica gel plate at intervals of 24 hours for 7 days, and the standard deviations of R_F values were found to be 0.011 and 0.007 for progesterone and testosterone, respectively, indicating good reproducibility of the method.

3.2. Validation of the method

Calibration curves were linear over the concentration range of 1–200 ng per spot for both steroid hormones, with correlation coefficients of 0.9988 and 0.9993 for progesterone and

Table 1 – Chromatographic parameters for the separation of progesterone and testosterone.

Plate	No. of days	ΔR_F	α	$R_{S,V}$	$R_{S,D}$
Plain silica gel	1	0.08	2.09	0.80	0.64
	2	0.05	1.81	0.54	0.41
	3	0.04	1.54	0.67	0.44
	4	0.06	2.06	0.60	0.30
	5	0.08	2.09	0.80	0.71
	6	0.07	1.66	0.67	0.45
	7	0.06	2.06	0.55	0.41
Au NPs-APTS modified silica gel	1	0.19	3.16	3.09	2.54
	2	0.24	4.04	3.23	2.73
	3	0.21	3.31	3.27	2.89
	4	0.20	2.86	2.46	1.96
	5	0.18	3.14	2.76	2.33
	6	0.17	3.31	2.83	2.21
	7	0.20	3.51	2.77	2.19

Au NPs-APTS = gold nanoparticles grafted 3-triethoxysilylpropylamine.

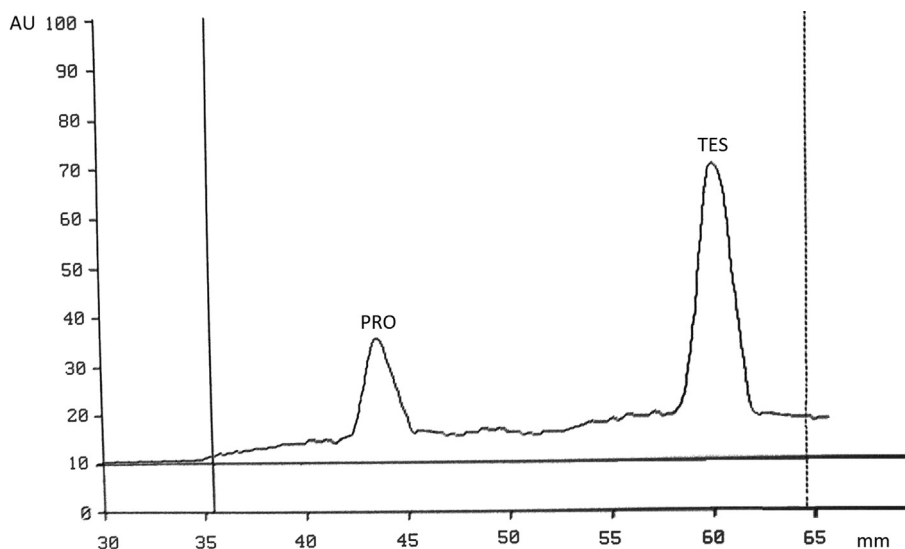


Fig. 4 – Densitograms of standard solution of progesterone and testosterone (50 ng per spot) on the Au NPs-APTS modified silica gel plate. Au NPs-APTS = gold nanoparticles grafted 3-triethoxysilyl propylamine; PRO = progesterone; TES = testosterone.

testosterone, respectively, confirming good linearity of the method. Furthermore, using Au NPs-APTS modified silica gel plate, the limits of detection of 0.16 ng and 0.13 ng per spot and the limits of quantification of 0.51 ng and 0.40 ng per spot were found for progesterone and testosterone, respectively. Chromatographic specificity was assessed on the basis of the retention factors and by comparing the densitograms. The steroids were identified by comparing the peak positions (R_f) using the chromatograms obtained from standard solutions containing the active substances with those obtained from sample solutions. As can be seen from Fig. 4 and 5, well-formed peak maxima were obtained, and the peaks appeared to be identical to those of the reference compounds.

The intra- and interday precisions, expressed as RSD%, were calculated for low (5 ng per spot), medium (50 ng per spot), and high (150 ng per spot) quality control levels in five replicates, and the results are presented in Table 2. As can be seen, the RSDs did not exceed 3.1% at any level, which indicates good precision of the method.

3.3. Application of the method

The applicability of the proposed method for determination of the two steroids in urine samples was also evaluated on both Au NPs-APTS modified and plain silica gel plates. As shown in Table 3, progesterone could not be detected on the both plates

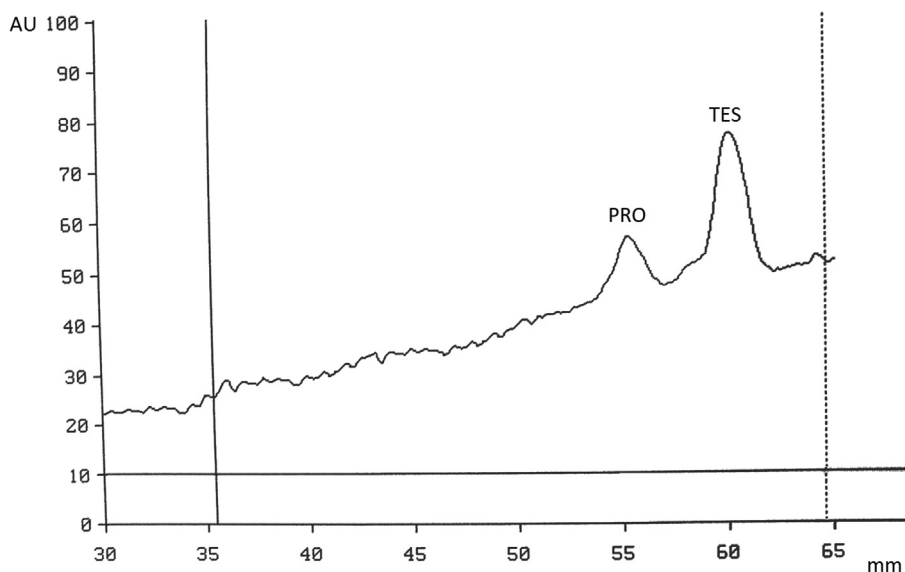


Fig. 5 – Densitograms of sample solution (urine) containing progesterone and testosterone (50 ng per spot) on the plain silica gel plate. PRO = progesterone; TES = testosterone.

Table 2 – Results from precision studies for determination of progesterone and testosterone on Au NPs-APTS modified silica gel plate ($n = 5$).

Concentration ^a	Measured concentration ^a		RSD (%)		Recovery (%)	
	Progesterone	Testosterone	Progesterone	Testosterone	Progesterone	Testosterone
Intraday precision						
5	4.9 ± 0.1	4.9 ± 0.1	1.9	2.7	98.2	98.8
50	49.1 ± 1.2	49.9 ± 1.1	2.4	2.1	98.2	99.7
150	146.2 ± 1.6	148.4 ± 2.6	1.5	1.8	97.4	98.9
Interday precision						
5	5.1 ± 0.1	4.9 ± 0.1	3.1	2.4	101.8	98.2
50	48.7 ± 1.2	49.3 ± 1.3	2.8	1.9	97.4	98.6
150	147.3 ± 1.9	149.0 ± 2.3	1.6	1.8	98.2	99.3

Au NPs-APTS = gold nanoparticles grafted 3-triethoxysilyl propylamine; RSD = relative standard deviation.

^a Nanogram per spot.

Table 3 – Results for the determination of progesterone and testosterone in urine samples (\pm RSD, $n = 3$).

Plate	Amount added ^a	Amount found ^a		Recovery (%)	
		Progesterone	Testosterone	Progesterone	Testosterone
Plain silica gel	0	0	1.22 ± 0.3	—	—
	10	9.68 ± 0.6	10.93 ± 0.8	96.8	97.5
	25	24.67 ± 1.3	27.24 ± 1.1	98.7	103.9
	50	49.65 ± 1.7	48.19 ± 0.7	99.3	94.1
Au NPs-APTS modified silica gel	0	0	1.27 ± 0.4	—	—
	10	9.91 ± 0.2	11.48 ± 0.4	99.1	101.9
	25	24.85 ± 0.3	25.95 ± 0.9	99.4	98.8
	50	50.39 ± 0.4	50.81 ± 1.2	100.7	99.1

Au NPs-APTS = gold nanoparticles grafted 3-triethoxysilyl propylamine; RSD = relative standard deviation.

^a Nanogram per spot.

within the detectable range, whereas testosterone was clearly detected ($R_F = 0.89$). When the urine samples were spiked with each steroid at concentration levels of 10 ng, 25 ng, and 50 ng per spot, the resultant samples produced two distinct spots at R_F values of 0.72 and 0.89 for progesterone and testosterone,

respectively, which matched well with the standards. The typical densitograms obtained from the spiked urine sample (50 ng per spot) on Au NPs-APTS modified and plain silica gel plates are shown in Figs. 5 and 6. As can be seen, baseline noises in the Au NPs-APTS modified silica gel plate are much

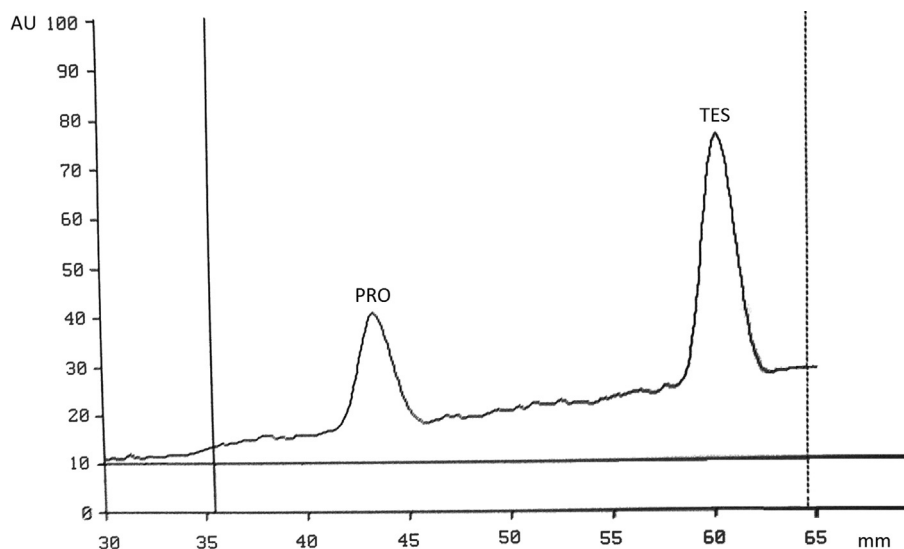


Fig. 6 – Densitograms of sample solution (urine) containing progesterone and testosterone (50 ng per spot) on the Au NPs-APTS modified silica gel plate. Au NPs-APTS = gold nanoparticles grafted 3-triethoxysilyl propylamine; PRO = progesterone; TES = testosterone.

lower than those existing in the plain silica gel plate. As mentioned previously, Au NPs-APTS modified silica gel can remove the interfering signals from the urinary proteins through their interactions with the Au NP surface. Thus, the proposed method can be useful for situations in which simple, fast, and sensitive analysis of the steroids in biomatrices must be performed with minimum matrix interferences.

4. Conclusion

In this study, the use of Au NPs-APTS modified silica gel as a suitable stationary phase for TLC separation has been demonstrated. The prepared stationary phase was applied for the separation and determination of progesterone and testosterone in human urine samples, and the results were compared with those obtained using plain (i.e., unmodified) silica gel plates. Some chromatographic parameters such as ΔR_F , R_S , α , and reproducibility of R_F were also evaluated. The experimental results indicate that the incorporation of Au NPs in the structure of conventional silica gel stationary phase can remove the baseline interfering signals from biomatrices, and the method can be useful for the analysis of clinical samples. In a word, the developed method can be used for rapid screening and sensitive determination of steroid hormones in complex biological matrices.

Conflicts of interest

There is no potential conflicts of interest.

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