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$Ru(phen)_3^{2+}$ doped silica nanoparticle based immunochromatographic strip for rapid quantitative detection of β-agonist residues in swine urine

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ABSTRACT

A $Ru(phen)_3^{2+}$ doped silica nanoparticle based immunochromatographic strip was developed for the rapid and quantitative detection of five common β-agonist (salbutamol (SAL), cimbuterol, terbutaline, clenbuterol, and brombuterol) residues in swine urine. The broad spectrum monoclonal antibodies generated by immunizing BALB/c mice with salbutamol conjugated cationic bovine serum albumin. The fluorescence intensities (FIs) of the strip on the test line (FI_T) and control line (FI_C) were determined using a strip reader. Parameters that influenced the antibody and antigen interaction on the test strip were investigated by recording FI_T and FI_C values, and the concept of FI_T/FI_C ratio was used to offset the inherent heterogeneity of the test strips and the effect of the sample matrix. Under optimal conditions, the linear range for the quantitative detection of SAL was 0.6–5.0 ng/ml with a half maximal inhibitory concentration at 1.78 ng/ml. The limit of detection for real swine urine was 0.43 ng/ml. The recovery rates of the intraassay for spiked urine at SAL concentrations of 0.8, 1.5, and 3.5 ng/mL were 88.06% ± 3.75%, 95.77% ± 5.33%, and 94.06% ± 7.43%, whereas those for the interassay were 84.69% ± 5.0%, 95.06% ± 9.3%, and 88.34% ± 7.71%, respectively. The developed quantitative method exhibited excellent agreement with a commercially available competitive enzyme-linked immunosorbent assay kit for SAL-spiked urine samples, with a correlation of coefficient of 0.95 and a slope of 0.99 ($n=36$). The results indicated that the developed test strip enables sensitive, reproducible, and easily implementable screening for the rapid and quantitative detection of β-agonist residues in swine urine.

1. Introduction

β-agonist residues in animal tissues can increase toxicological risks to consumers [1,2]. Thus, many classical confirmatory methods, including liquid chromatography [3–7], gas chromatography [8,9], liquid chromatography–mass spectrometry [10–13], and gas chromatography–mass spectrometry [14–16], have been developed to monitor the illicit use of such residues. These methods are highly sensitive, reliable, and widely used, but require extensive sample preparation and skilled analysts to operate complicated instruments. Moreover, these techniques entail high costs and time-consuming procedures [17].

An immunochromatography test strip (ICTS) is a popular screening tool for conducting onsite testing because of its acceptable sensitivity, user-friendliness, and rapidity (5–10 min). Gold nanoparticles, a type of metal nanoparticle-based sensors, are popular tools for detecting various types of β-agonists [18]. However, its performance depends on the amount of molecules gathered; it is also susceptible to optical interference, thereby exhibiting relatively low sensitivity and inaccurate qualitative or semiquantitative results. Quantum dots and dye doped nanoparticles are representative fluorescent nanoparticle probes of increasing research focus [19,20]. Dye doped nanoparticles vary in diameter from 2 to 200 nm, contain a large quantity of dye molecules embedded in a polymer or silica matrix, and emit more intense fluorescence signals than do organic fluorophores [21]. Their excellent brightness makes them especially suitable for trace bioanalysis without additional reagents or signal amplification steps [22]. Compared with polymer nanoparticles, fluorescent silica nanoparticles (FSNPs) possess several advantages, such as easy surface modification and solution treatment processes, and simple separation through centrifugation during particle preparation because of the high density of silica [21]. Silica nanoparticles are more hydrophilic and biocompatible; they do not suffer from microbial degradation and maintain their stable structure even with environmental changes (e.g., changes in pH). Thus, FSNPs

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present promising potential for use in various biological applications and are predicted to be ideal reporters for fluorescence-based ICTS because of the aforementioned advantages and excellent photo-physical properties.

In this study, $Ru(phen)_3^{2+}$ doped silica nanoparticles as fluorescent reporters were introduced into an immunochromatographic test strip for rapid, sensitive, quantitative, and broad spectrum screening detection of five common β-agonist (salbutamol (SAL), cimbuterol, terbutaline, clenbuterol, and brombuterol) residues in swine urine. The effects of immunoreaction time, pH, and ionic strength on the antibody and antigen interaction of the strip were analyzed. The fluorescence intensities (FIs) of the test line (FI_T) and control line (FI_C) were measured and the ratio of FI_T/FI_C was set to offset the interference from sample matrix and inherent heterogeneity of the test strips [19,23]. The performance of the proposed quantitative method, including its half maximal inhibitory concentration (IC_{50}), limit of detection (LOD), detection range, precision, and reliability, were evaluated. Experimental results revealed that the $Ru(phen)_3^{2+}$-doped silica nanoparticle-based immunochromatographic test strip has satisfactory sensitivity, as well as acceptable accuracy and precision.

2. Experimental

2.1. Reagents and materials

All β-agonists, namely salbutamol (SAL), cimbuterol, terbutaline, clenbuterol, cimaterol, ractopamine, mabuterol, bambuterol, tulobuterol, clorprenaline, penbutolol, and brombuterol; 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC · HCl); N-hydroxysulfosuccinimide (NHSS); bovine serum albumin (BSA); Freund's complete adjuvant (FCA); and Freund's incomplete adjuvant (FIA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Cationic BSA (CBSA) was provided by ShiYi Biotechnology, Inc. (Shanghai, China). Goat-anti-mouse IgG and the enzyme-linked immunosorbent assay (ELISA) kit for SAL were provided by Wuxi Zodoboer Biotech. Co., Ltd. (Wuxi, China). Dichlorotris (1, 10-phenanathroline) ruthenium (II) hydrate ($Ru(phen)_3^{2+}$) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Tetraethoxysilane (TEOS) was obtained from Tiantai Chemical, Int. (Tianjin, China) and distilled under reduced pressure before use. The nitrocellulose membrane, sample pad, conjugate release pad, and absorbent pad were obtained from Millipore (Bedford, MA, USA). Ultra-pure water was prepared by Elix-3 and Milli-QA (Molsheim, France). All other reagents are of analytical grade and purchased from Sinopharm Chemical Corp. (Shanghai, China).

Swine urine samples, ascertained to be free of SAL, cimbuterol, terbutaline, clenbuterol, and brombuterol by liquid chromatography–tandem mass spectrometry (LC-MS/MS), were obtained from different local farms. The blank urine mixture was obtained by combining 20 randomly selected urine samples and stored at −20 °C for later use. All swine urine samples used for the strip analysis were centrifuged at 8000*g* for 5 min to remove any precipitate. SAL stock solution was prepared by dissolving 1.0 mg SAL in 1.0 ml methanol. The fortified urine samples (0–5 ng/ml of SAL) were prepared by spiking a stock solution into a blank-mixture urine sample.

2.2. Apparatus

The microplate reader (DNM9602) was from Perlong Instruments, Ltd. (Beijing, China). The Bio-Dot XYZ platform and CM 4000 cutter were invented by Bio-Dot (Irvine, CA).

2.3. Preparation of immunogen and coating antigen

The SAL succinate was synthesized by the mixed anhydride method as previously described with minor modifications [24], and the immunogen of the SAL–CBSA conjugate was prepared by an ester activation method [25]. In brief, 20 mg SAL in 4.0 ml anhydrous ethanol was mixed with 9 mg succinic anhydride in 1 ml anhydrous toluene. After the mixture was stirred at room temperature for 2 h, the carboxyl SAL was isolated by centrifugation at 8000*g* for 10 min and washed with anhydrous ethanol three times. Then, 5 mg of carboxyl SAL, 2.5 mg of EDC · HCl, and 2.0 mg of NHSS were mixed with 20 mg of CBSA and BSA in 2 ml of 0.01 M phosphate buffer saline (PBS, pH 5.5), and stirred at 4 °C overnight. The SAL–CBSA and SAL–BSA conjugates were dialyzed against 0.01 M in pH 7.4 PBS to remove free hapten.

2.4. Production of anti-SAL monoclonal antibodies

Four eight-week old female BALB/c mice were subcutaneously injected at multi-points with SAL–CBSA conjugates. The first dose consisted of 100 μg of immunogen in 0.1 ml PBS and mixed with an equal volume of FCA. Four subsequent injections were performed every 3 weeks with the same dosage of immunogen emulsified in FIA. Ten days after the fifth immunization, the sensitivity of the antiserum was determined by an indirect competitive ELISA (icELISA). The mouse with serum that showed the highest inhibition was given the sixth boost injection. Fusion was performed on the third day after the last injection. Twelve days later, the culture supernatant was tested with ciELISA to screen antibody-producing cells. Stable hybridomas-secreting anti-SAL monoclonal antibodies (anti-SAL mAbs) were selected and cloned by limited dilution. Ascitic fluid was produced in paraffin-primed BALB/c mice. Antibodies were purified from this fluid using ammonium sulfate precipitation [26].

2.5. Preparation and characterization of FSNPs

The FSNPs were prepared according to a previously reported procedure [27,28]. Briefly, 2.4 ml TEOS was added to an ethanol solution (30 ml) containing 1.0 ml ammonia and 1.125 ml pure water. The reaction mixture was kept at 25 °C and stirred for 12 h; 0.5 ml of $Ru(phen)_3^{2+}$ ethanol solution (1 mg/ml) was added at 3 h reaction time. The $Ru(phen)_3^{2+}$ doped silica nanoparticles were further functionalized with the amino group by adding 0.3 ml of (3-aminopropyl) triethoxysilane under vigorous stirring for 12 h. The resultant nanoparticles were isolated by centrifugation at 15,000*g* for 10 min and washed with 10 ml ethanol three times. The $Ru(phen)_3^{2+}$ doped SiO_2–NH_2 was resolved in 15 ml DMF solution and then added dropwise to 20 ml of 0.1 M succinic anhydride. The mixture was stirred for 24 h. The carboxyl group modified $Ru(phen)_3^{2+}$ doped silica nanoparticles (FSNPs) were cleaned as described above and kept in pure water for later use. Transmission electron microscopy (JEM-2100, JEOL, Japan) was performed to characterize the diameter of the FSNPs.

2.6. Preparation and characterization of mAb-labeled FSNPs

The mAb-labeled FSNPs (FSNPs–mAbs) were prepared according to previous methods with some modifications [29]. Briefly, 1 mg of FSNPs, 0.75 mg of EDC · HCl, and different volumes (30, 40, and 50 μl) of anti-SAL mAbs (1.0 mg/ml) were added to 2 ml of 0.02 M PB solution (pH 5.0) with a magnetic stirrer. After reaction at room temperature for 2 h, the mixture was blocked with 200 μl of 10% BSA (w/v) for 30 min and then separated by centrifugation at 7500*g* for 5 min. The FSNPs–mAbs were washed twice with 2 ml of 0.02 M PB (pH 5.0) and then resuspended in a 200 μL solution containing

0.02 M Na_2HPO_4, 5% sucrose (w/v), 3% trehalose (w/v), 0.1% NaN_3, and 0.05% PEG 20000. The solution was stored at 4 °C for later use. The average hydrodynamic diameters and fluorescent intensities of the resultant conjugates (0.5 mg/ml) were characterized using a nanoparticle size analyzer (Malvern Instruments, Ltd., UK) and a Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Ltd., Japan), respectively.

2.7. FSNP-based strip assay

2.7.1. Preparation of FSNP-based test strip

The test strip was prepared as previously described with some modifications [30,31]. The sample pad was soaked in 0.1 M of PBS (pH 7.2) solution containing 0.5% Tween-20, 0.5% BSA, 3% sucrose, and 0.05% sodium azide, and then dried at 60 °C for 2 h. The conjugate pad was pretreated with 0.01 M PBS (pH 7.2) containing 0.25% Tween-20 and 4% sucrose for 24 h and then dried at 60 °C for 2 h. The FSNP–mAbs solution was jetted into a treated conjugate pad at densities of 3, 4, and 5 μl/cm, and then dried with a vacuum dryer at 37 °C for 2 h. The donkey-anti-mouse antibody (1.0 mg/ml) and SAL–BSA conjugate (0.15, 0.3, and 0.5 mg/ml) were immobilized on the NC membrane as the control and test lines, respectively, and dried at 35 °C for 3 h. The sample pad, conjugate pad, NC membrane, and absorption pad were assembled, and the laminated sheet was attached onto a plastic backing plate and then cut into 4-mm wide individual strips (Fig. 1A). All the strips were packaged in a plastic bag containing a desiccant gel and stored at room temperature.

2.7.2. Test procedure and parameter optimization

As presented in Fig. 1B, the values of FI_T, FI_C and FI_T/FI_C ratio were recorded by a commercial fluorescent strip reader (Huguo Science Instrument Co., Ltd., Shanghai, China), which uses a high power 450 nm LED arrays as excitation light source, the emission fluorescence of the FSNPs on the test and control lines were focused with a cylindrical lens and received by a light sensitive cell. When the analyte was excluded from the sample, the FSNPs–mAbs were captured by the coating antigen and goat-anti-mouse IgG to form a fluorescent signal on the test and control lines. When the analyte was included in the sample, it competed against the antibody on the surface of the FSNPs with coating antigen on the test line, thereby changing the fluorescence intensity (strong to weak) on the line. This effect disappeared when the analyte completely blocked the antibody on the FSNPs of the test strip. Thus, the development of fluorescence intensities on the test and control lines can indirectly reflect the dynamic interaction between antibody (FSNPs–mAbs on conjugate pad) and antigen (SAL–BSA on the test line and goat anti-mouse IgG on control line) interactions. The antibodies and antigens interactions were influenced by pH, salt concentration of the sample solution, and immunoreaction time. These parameters were optimized by using 0.01 M of PBS solution as a model system. Samples with different pH values (5–9) were prepared by adding 0.1 M of HCl or 0.1 M NaOH to the buffer solution. Samples with various ionic strengths (0, 0.1, 0.3, 0.5 M) were obtained by adding a series of concentrations of NaCl to pH 5.0 PBS. Under the optimized reaction pH and salt concentration of the buffer solution, the optimal immunoreaction time was obtained through the kinetic curves that were drawn by plotting FI against time under different spiked concentrations (0, 1, 2, 3 ng/ml) for FSNPs–mAbs and SAL–BSA, as well as for FSNPs–mAbs and anti-mouse IgG. The optimization was performed as follows. An 80 μl aliquot of the buffer solution was pipetted into the strip sample. After 1 min of incubation, the strip was inserted into the strip reader. FI_T and FI_C were recorded every 20 s for 30 min and repeated three times for each sample.

2.7.3. Quantitative standard curve of the strip

Serial standard solutions were prepared by diluting the SAL stock solution with a blank-mixture urine sample to a final concentration of 0 (as negative control), 0.6, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 ng/ml. The FI_T/FI_C of the strip was measured with a test strip reader after adding a sample for 15 min. The FI_T/FI_C of the

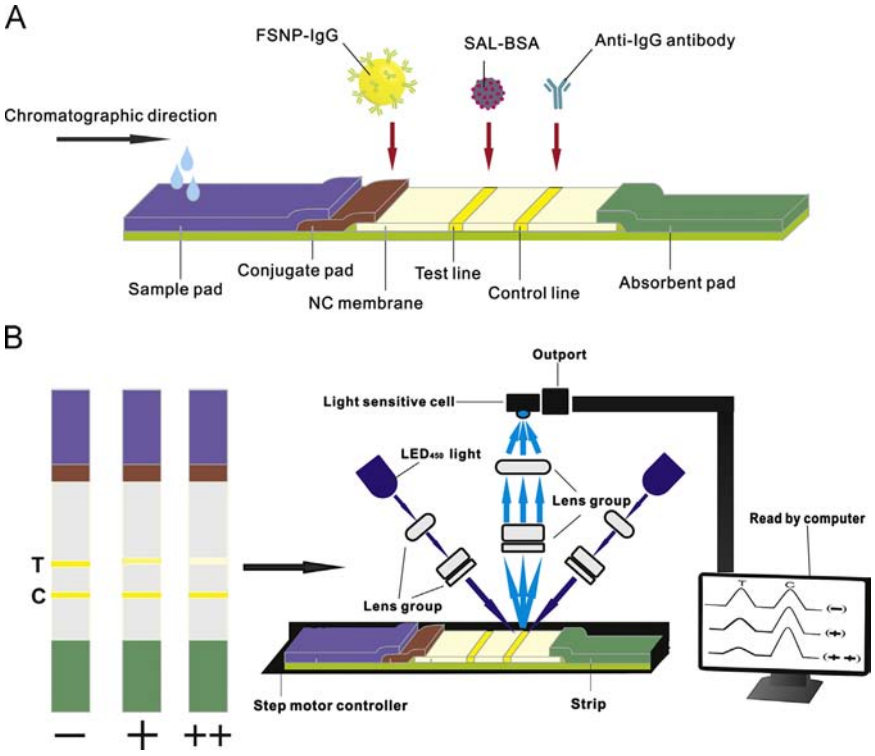


Fig. 1. (A) schematic illustration of the test strip and (B) detection of SAL using FSNP-based ICTS.

negative control and positive samples were designated as B_0 and B, respectively. The standard curve was constructed by plotting the B/B_0 values against the logarithm of the SAL concentrations.

2.7.4. Assay validation

The LOD of the real sample was calculated as the mean determined concentration of 20 randomly negative swine urines plus threefold standard deviation [24]. The accuracy and precision of the strip were evaluated by analyzing the recovery and the intra- and inter-assay coefficient of variation. The intra-assays were estimated by one batch of the test strips for five replicate swine urine samples spiked with the target analyte with concentrations of 0.8, 1.5, and 3.5 ng/ml, representing low, medium, and high levels of the SAL residues, respectively. The inter-assays were performed every 10 days for 30 sequential days. Spiked samples (36) were prepared for ELISA and test strip comparative assay, with concentrations of 1.0–4.5 ng/ml. The samples were analyzed in duplicate using the test strip and ELISA methods. The correlation analysis of the results of the two methods was used to evaluate their quantitative concordance.

3. Results and discussion

3.1. Characterization of immunogen and anti-SAL monoclonal antibody

The derivate of SAL was confirmed by MS and nuclear magnetic resonance (NMR) (Fig. S1A and B). The immunogen was synthesized according to an ester activation method to couple the carboxyl group of SAL succinate with the amino group of CBSA. The coupling ratio of the conjugate reached at 19:1 (Fig. S2) due to the CBSA owned more amino groups on the surface in comparison with native BSA. Additional, CBSA can induce a more rapid immunological response because of it containing a positive charge on the surface bound to antigen presenting cell in vivo faster than native BSA [32]. For anti-SAL antibody production, the spleen cells of the injected mouse, in which the titer of the anti-serum reached 1:80,000, were fused with SP2/0 myeloma cells after the last booster injection. The positive hybridoma cell lines were screened using icELISA and one hybridoma clone, which produced a high titer anti-SAL antibody after four subclones by limited dilution method. The antibody subtyping was identified as IgG_1 subclass with a mouse monoclonal antibody isotyping kit.

3.2. Characterization of FSNPs and FSNP–mAbs conjugates

The FSNPs–mAbs were obtained by coupling the amino group of anti-SAL mAbs with the carboxyl group on the surface of the FSNPs. The Hitachi F-4500 fluorescence spectrophotometer and nanoparticle size analyzer were used to characterize the fluorescence properties and hydration diameter of the FSNPs with/without antibodies, respectively. The maximum emission wavelength of the FSNPs–mAbs did not shift compared with the free FSNPs, and fluorescence intensity slightly decreased (Fig. 2A) presumably because of the antibody on the surface of the nanoparticles, which covered part of the fluorescence. Fig. 2B shows the transmission electron microscopic results of the FSNPs prepared by doping Ru $(phen)_3^{2+}$ into the silica nanoparticle. The FSNPs are uniform in size distribution, with an average diameter of 86 nm. Fig. 2C shows that the average hydrodynamic diameter of the free FSNPs is about 200 nm, whereas that of the FSNPs–mAbs increased to about 220 nm (the hydrodynamic diameter of mAbs is 8–10 nm). The results confirmed that the anti-SAL mAbs were successfully coupled on the surface of the FSNPs and that the FSNPs–mAbs maintained good mono-dispersibility. These findings indicate that the FSNPs–mAbs have good applicability.

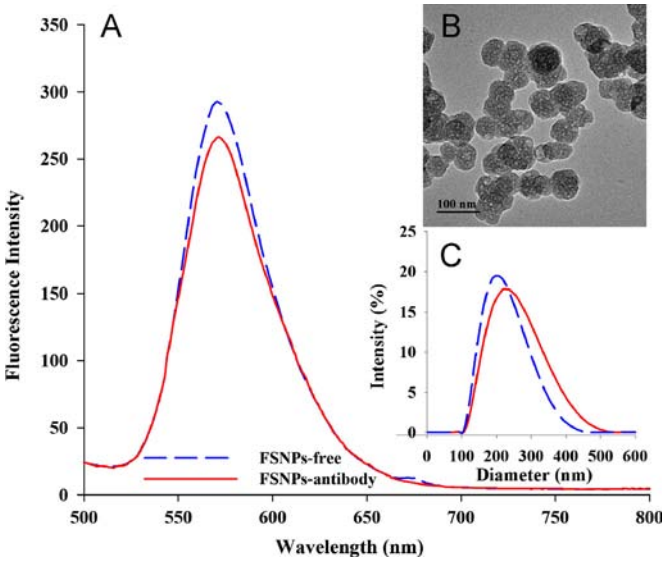


Fig. 2. Characterization of the free FSNPs and FSNP–mAbs conjugates. (A) Photoluminescence spectra of FSNPs with/without antibody labeled, (B) transmission electron microscopy image of the resultant. FSNPs and (C) hydrodynamic diameter of FSNPs with/without antibody labeled.

3.3. Optimization of the FSNP-based test strip and immunoassay conditions

The amount of antibody labeled with FSNPs, the concentration of SAL–BSA on the test line, and the jetted volume (μl/cm) of the FSNPs–mAbs on the conjugate pad were the most important factors that affected the sensitivity of the strip and the fluorescence intensity of the test line. An orthogonal L9 $(3)^3$ test that includes all the above-mentioned factors was designed to optimize the parameters of the FSNP-based test strip. The analysis results of the orthogonal test are presented in Table 1, which shows that the optimal combination was as follows: 40 μg mAbs labeled with 1 mg free FSNPs, 3 μl/cm of the volume of FSNP–mAbs, and 0.3 mg/ml SAL–BSA. Under these conditions, the means of FI_T and FI_C were 683 and 491, respectively, and the inhibition rate of the strip for the 2 ng/ml spiked samples was the highest (35.6%).

The effect of pH value and ionic strength of sample solution on the sensitivity of the strip was shown in Fig. 3. With the pH decreased from 9 to 5, the FI_T and ratio of FI_T/FI_C significantly increased from 1177 ± 38 to 1577 ± 22 and 1.25 ± 0.03 to 2.23 ± 0.01, respectively (Fig. 3A). The above result indicated that the mAb and SAL interaction was enhanced in weak acidic buffer (pH 5.0) but suppressed under neutral or basic conditions (pH 7.0–9.0). The same trend was observed in the SAL-spiked test (data not shown). Thus, pH 5.0 was selected as the working pH. Under the different NaCl concentrations (0–0.5 M), the strip test results showed the FI_T, FI_C and FI_T/FI_C ratio values exhibited almost constant throughout the experiment indicated that the ionic strength in the sample did not significantly affect the analytical performance of the strip (Fig. 3B).

In the strip test, the FI development of the test and control lines was also affected by the concentration of the analyte associated with an appropriate immunoreaction time. The immunological kinetics under different SAL concentrations was evaluated by monitoring FI_C and FI_T development during the 30-min incubation. As illustrated in Fig. 4A and B, the FI_C and FI_T sharply increased in the first 10 min and then maintained slow growth in the subsequent 20 min. The FI_T/FI_C ratio was large at 12 min after the

sample addition and remained constant after 15 min. As indicated by these findings, the optimal incubation time was 15 min. When higher concentrations of SAL were present in the sample, the constant FI_T/FI_C ratio was reached in 7–12 min (pink arrows in Fig. 4C), which indicates that the FI_T/FI_C ratio can eliminate the effects of the dynamic differences in immunoreaction and shorten strip interpretation time [33].

3.4. Assay validation

To decrease the interference from swine urine, a pH 5.0 blank mixture urine sample was used to dilute the stock standard solution. The standard curve for the detection of SAL showed a linear range between 0.6 and 5.0 ng/ml with an IC_{50} at 1.78 ng/ml. The regression equation was $y=-0.247\mathrm{Ln}(x)+0.6456$ (Fig. 5), with a reliable correlation of coefficient ($R^2=0.9941$). The LOD of SAL was calculated as 0.43 ng/ml according to the mean plus threefold standard deviations of the measured concentration of 20 blank

Table 1
The analysis results of the orthogonal test (SAL spiked in negative swine urine).

No.	The concentration (μg/mg) of labeled antibody on FSNPs	The jetted volume (μl/cm) of FSNPs probe	Antigen concentration (mg/ml)	The FI of test and control lines[a]	The inhibition rate of positive sample[b] (%)
1	30	3	0.15	413/T, 515/C	15.6
2	30	4	0.30	596/T, 419/C	17.5
3	30	5	0.50	998/T, 409/C	25.7
4	40	3	0.30	683/T, 491/C	35.6
5	40	4	0.50	1259/T, 361/C	18.9
6	40	5	0.15	548/T, 691/C	7.5
7	50	3	0.50	994/T, 283/C	17.2
8	50	4	0.15	594/T, 526/C	18.9
9	50	5	0.30	882/T, 408/C	12.5

[a] The mean of FI_T and FI_C values were obtained from the negative sample.
[b] The inhibition rates were obtained from 2 ng/ml SAL spiked sample.

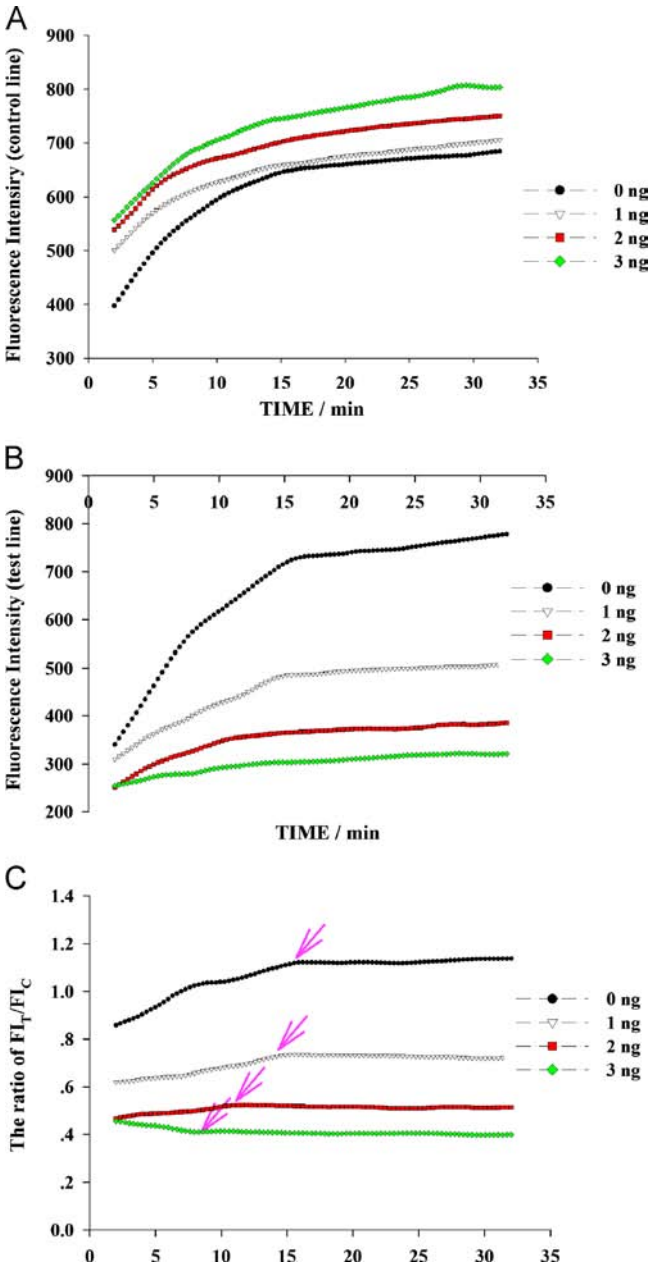


Fig. 4. Immune reaction kinetics curves with different SAL concentrations. (A) Control line, (B) test line, and (C) ratio of FI_T/FI_C.

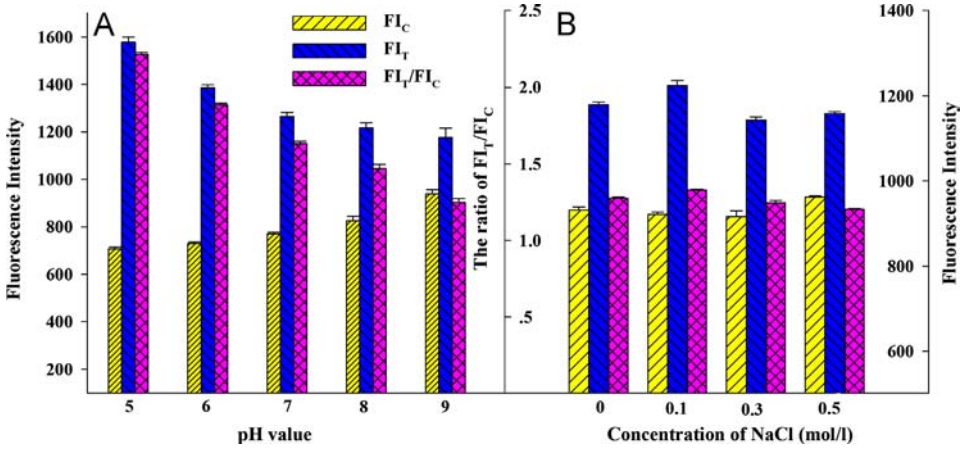


Fig. 3. The effects of the ionic strength and pH value on the FI_T, FI_C and the ratio of FI_T/FI_C. (A) in different pH values (5–9), (B) in different pH values in different concentrations of NaCl (0, 0.1, 0.3, 0.5 M). Error bars are based on three duplicate measurements.

swine urine samples, which were ascertained to be free of β-agonists by LC–MS/MS. The cross-reaction assay was evaluated by running 12 structurally related β-agonists according the following equation: $Cr\% = (IC_{50\ SAL})/(IC_{50\ analog})100\%$ [29]. The results (Table 2) indicated the strip exhibited a strong cross-reacting with cimbuterol (76.4%), terbutaline (55.1%), clenbuterol (42.7%), and brombuterol (34.4%) while negligible cross-reactivity ($< 1\%$) with ractopamine, mabuterol, bambuterol, tulobuterol, clorprenaline, cimaterol, and penbutolol. The above result indicated that the developed FSNP-based strip can be used for broad spectrum screening of five β-agonist residues in swine urine. However, cross-reactions increase the predicted concentration of the target compound and the potential of false-positive when both the target and structurally similar compounds are present. Thus, all positive results should be further verified with confirmed methods to ensure their accuracy especially applied in the detection of specific compound.

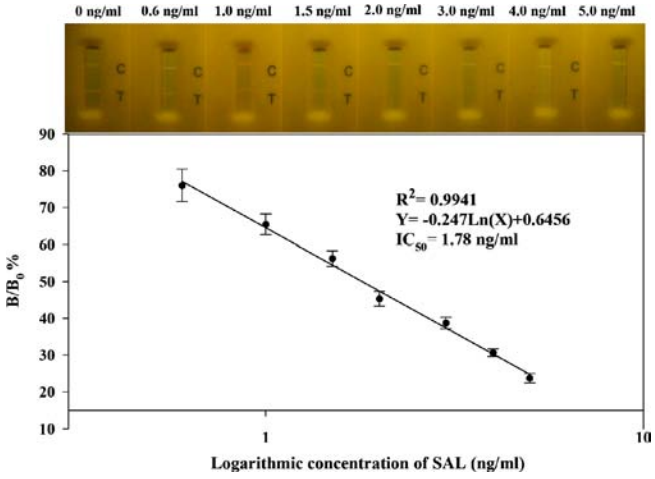


Fig. 5. Calibration curves for SAL quantitative analysis ($n=5$). Upper is the real strip results observed by a LED_{450} lamp under different SAL standard solutions.

The accuracy and precision of the test strip were evaluated through recovery studies by using SAL-spiked swine urines. The results are shown in Table 3. The average recoveries for the intra-assay ranged from 88.06% to 95.77%, with a relative standard deviation ranging from 4.2% to 7.9%. The inter-assay recoveries ranged from 84.69% to 95.06%, with a relative standard deviation ranging from 5.9% to 9.8% at SAL concentrations between 0.8 and 3.5 ng/ml. The variation within intra- and inter-assay recoveries below 10% demonstrated an acceptable level of precision for SAL strip quantification. The 36 spiked swine urines with SAL in the range 1.0–4.5 ng/ml were determined using the strips and a commercially available SAL ELISA kit. The recoveries of the test strip for 36 actual positive samples were 74–100%, and those of the ELISA kit was 77–106% (Fig. 6). The two methods exhibited highly significant correlation and strong agreement, as reflected by a correlation coefficient (R^2) of 0.95 and a slope of 0.99.

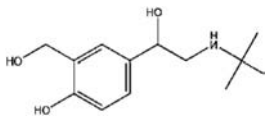
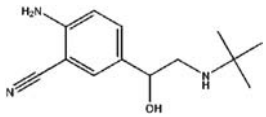
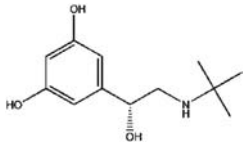
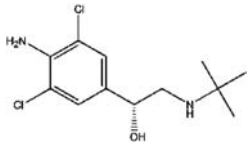
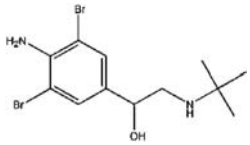
4. Conclusion

This work described a rapid, sensitive, and quantitative immunochromatographic strip for broad spectrum screening of SAL, cimbuterol, terbutaline, clenbuterol, and brombuterol residues in swine urine. The $Ru(phen)_3^{2+}$ doped silica nanoparticles were

Table 3
The accuracy and precision of the test strip in SAL spiked swine urine samples.

Spiked SAL (ng/ml)	Intra-assay				Inter-assay			
	Mean	Recovery (%)	SD	CV%	Mean	Recovery (%)	SD	CV%
0.8	0.704	88.06	0.03	4.2	0.677	84.69	0.04	5.9
1.5	1.437	95.77	0.08	5.6	1.426	95.06	0.14	9.8
3.5	3.292	94.06	0.26	7.9	3.092	88.34	0.27	8.7

Table 2
Linear relationships and sensitivity of β-agonists.

Compounds	Structure	Linear range (ng/ml)	Linear equation	R^2	IC_{50} (μg/kg)	LOD
Salbutamol		0.6–5.0	$y=-0.247\ln x+0.6456$	0.9941	1.78	0.43
Cimbuterol		1.4–5.0	$y=-0.371\ln x+0.8138$	0.9931	2.33	1.32
Terbutaline		1.6–8.5	$y=-0.292\ln x+0.8424$	0.9956	3.23	1.44
Clenbuterol		2.0–12	$y=-0.266\ln x+0.8798$	0.9818	4.17	1.63
Brombuterol		3.0–13	$y=-0.282\ln x+0.9649$	0.9919	5.18	2.21

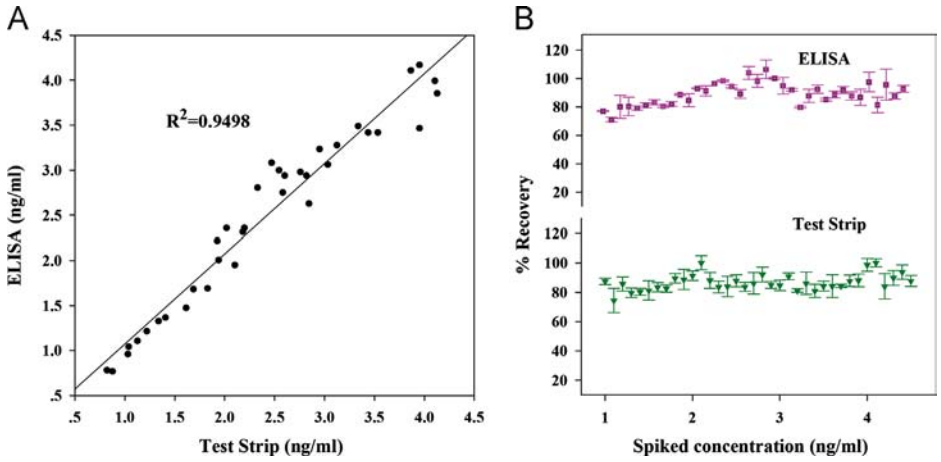


Fig. 6. (A) Method comparison between a FSNP-based test strip and a commercially available ELISA kit in 36 spiked real swine urines for SAL concentrations in the range of 1.0–4.5 ng/ml and (B) recovery of 36 spiked samples with test strip and ELISA. Error bars are based on two duplicate measurements.

successfully used in the strip test as fluorescent reporters. The ratio of FI_T/FI_C was introduced to offset the effects of the inherent heterogeneity of the strips and matrix. The performance of the developed quantitative method was evaluated and its reliability was validated by comparison with the commercial ELISA kit. The FSNP-based strip system using the FI_T/FI_C ratio is suitable for screening multiplexed β-agonist residues in swine urine.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.04.013.

References

[1] G.A. Mitchell, G. Dunnavan, J. Anim. Sci. 76 (1998) 208–211.
[2] M.J.F. Navarro, Lancet 336 (1990) 1311.
[3] L.A. Lin, J.A. Tomlinson, R.D. Satzger, J. Chromatogr. A 762 (1997) 275–280.
[4] E. Shishani, S.C. Chai, S. Jamokha, G. Aznar, M. Hoffman, Anal. Chim. Acta 483 (2003) 137–145.
[5] P.A. Guy, M. Savoy, R.H. Stadler, J. Chromatogr. B 736 (1999) 209–219.
[6] M.P. Turberg, T.D. Macy, J.J. Lewis, M.R. Coleman, J. AOAC Int. 78 (1995) 1394–1402.
[7] Y.T. Zhang, Z.J. Zhang, Y.H. Sun, Y. Wei, J. Agric. Food Chem. 55 (2007) 4949–4956.
[8] B. Bocca, M. Fiori, C. Cartoni, G. Brambilla, J. AOAC Int. 86 (2003) 8–14.
[9] L. He, Y. Su, Z. Zeng, Y. Liu, X. Huang, Anim. Feed Sci. Tech. 132 (2007) 316–323.
[10] G.J. Murray, J.P. Danaceau, J. Chromatogr. B 877 (2009) 3857–3864.
[11] B. Shao, X.F. Jia, J. Zhang, J. Meng, Y.N. Wu, H.J. Duan, X.M. Tu, Food Chem. 114 (2009) 1115–1121.
[12] B. Buszewski, T. Welerowicz, E. Tegowska, T.F. Krzeminski, Anal. Bioanal. Chem. 393 (2009) 263–272.
[13] J. Blanca, P. Munoz, M. Morgado, N. Mendez, A. Aranda, T. Reuvers, H. Hooghuis, Anal. Chim. Acta 529 (2005) 199–205.
[14] P. Gallo, G. Brambilla, B. Neri, M. Fiori, C. Testa, L. Serpe, Anal. Chim. Acta 587 (2007) 67–74.
[15] W. Liu, L. Zhang, Z.Y. Wei, S.P. Chen, G.N. Chen, J. Chromatogr. A 1216 (2009) 5340–5346.
[16] L.Q. Wang, Z.L. Zeng, Y.J. Su, G.K. Zhang, Z.P. Liang, L.M. He, J. Agric. Food Chem. 60 (2012) 6359–6363.
[17] K.E.I. Vanoosthuyze, C.J.M. Arts, C.H.V. Peteghem, J. Agric. Food Chem. 45 (1997) 3129–3137.
[18] J.Q. Jiang, Z.L. Wang, H.T. Zhang, X.J. Zhang, X.Y. Liu, S.H. Wang, Anal. Chem. 83 (2011) 6988–6955.
[19] Q. Yang, X. Gong, T. Song, S. Zhu, Y. Li, Y. Cui, Y. Li, B. Zhang, J. Chang, Biosensors. Bioelectron 30 (2011) 145–150.
[20] I.L. Medintz, H.T. Uyeda, E.R. Goldman1, H. Mattoussi, Nat. Mater. 4 (2005) 435–446.
[21] X. Zhao, R.P. Bagwe, W. Tan, Adv. Mater. 16 (2004) 173–176.
[22] L. Wang, K. Wang, S. Santra, X.J. Zhao, L.R. Hilliard, J.E. Smith, Y. Wu, W.H. Tan, Anal. Chem. 78 (2006) 646–654.
[23] L. Anfossi, M. Calderara, C. Baggiani, C. Giovannoli, E. Arletti, G. Giraudi, Anal. Chim. Acta 682 (2010) 104–109.
[24] Y.C. Lei, Y.F. Tsai, Y.T. Tai, C.Y. Lin, K.H. Hsieh, T.H. Chang, S.Y. Sheu, T.F. Kuo, J. Agric. Food Chem. 56 (2008) 5494–5499.
[25] D. Hughes, J. Beesley, Methods Mol. Biol. 80 (1998) 275–282.
[26] C.H. Xie, F.J. Chen, T.B. Yang, Anal. Biochem. 426 (2012) 118–125.
[27] Y.Q. An, M. Chen, Q.J. Xue, W.M. Liu, J. Colloid Interface Sci. 311 (2007) 507–513.
[28] D.W. Zhang, Z.Z. Wu, J.Q. Xu, J.L. Liang, J. Li, W.S. Yang, Langmuir 26 (2010) 6657–6662.
[29] H.M. Li, W.H. Lai, H.Y. Xu, B. Xu, W. Luo, H. Wei, S.X. Wang, Y.H. Xiong, Chin. J. Anal. Chem. 39 (2011) 1647–1652.
[30] W.H. Lai, Y. Xu, D.Y.C. Fung, Y.H. Xiong, Asia Pac. J. Clin. Nutr. 16 (2007) 106–110.
[31] X.L. Chen, H.Y. Xu, W.H. Lai, Y. Chen, X. Yang, Y.H. Xiong, Food Addit. Contam. A 29 (2012) 383–339.
[32] F. Welfringer, P. d'Athis, J.M. Scherrmann, F. Herve, J. Immunol. Methods. 307 (2005) 82–95.
[33] J.S. Ahn, S. Choi, S.H. Jang, H.J. Chang, J.H. Kim, K.B. Nahm, S.W. Oh, E.Y. Choi, Clin. Chim. Acta 332 (2003) 51–59.