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ORIGINAL PAPER

Solid Substrate–Room Temperature Phosphorimetry for the Determination of Trace Terbutaline Sulfate Based on Its Inhibition Oxidation of Rhodamine 6G by Sodium Periodate

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Abstract When 1.00 mol l^{-1} I^- is used as ion perturber, rhodamine 6G (Rh 6G) can emit strong and stable room temperature phosphorescence (RTP) on filter paper substrate in $KHC_8H_4O_4$–HCl buffer solution (pH=3.50), heated at 70 °C for 10 min. $NaIO_4$ can oxidize Rh 6G, which makes the RTP signal quench. Terbutaline sulfate (TBS) can inhibit $NaIO_4$ from oxidizing Rh 6G, which makes the RTP signal of Rh 6G enhance sharply. The content of TBS is linear correlation to ΔIp of the system. Based on the facts above, a new inhibition solid substrate-room temperature phosphorimetry (SS-RTP) for the determination of trace TBS has been established. The linear range of this method is 0.0104–2.08 pg $spot^{-1}$ (corresponding concentration: 0.026–5.2 ng ml^{-1}, with a sample volume of 0.4 μl) with a detection limit (L.D.) of 2.6 fg $spot^{-1}$ (corresponding concentration: 6.5×10^{-12} g ml^{-1}), and the regression equation of working curve is ΔIp=2.040+54.54 m_{TBS} (pg $spot^{-1}$), n=6, correlation coefficient is 0.9994. For the samples containing 0.0104 pg $spot^{-1}$ and 2.08 pg $spot^{-1}$ TBS, the relative standard deviation (RSD) are 3.8% and 2.3% (n=8), respectively, indicating good precision. This method has been applied to determination of trace TBS in the practical samples with satisfactory results. The reaction mechanism of $NaIO_4$ oxidizing Rh 6G to inhibit SS-RTP for the determination of trace TBS is also discussed.

Keywords Terbutaline sulfate · Rhodamine 6G · Inhibition solid substrate–room temperature phosphorimetry

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Introduction

Terbutaline sulfate (TBS), $((C_{12}H_{19}NO_3)_2{\cdot}H_2SO_4$, M= 548.66), was a kind of drugs used in the treatment of asthma, chronic obstructive pulmonary, emphysema, and other lung diseases [1]. When high doses of TBS are administered, it would stimulate the central nervous system and some anabolisms, so the administration of TBS was prohibited by the International Olympic Committee [2]. Therefore, searching for a new, sensitive and accurate method to determine trace TBS has been a new task which researchers both at home and aboard have dedicated to. Though many methods for the determination of trace TBS have been reported, such as *micro flow sensor* (detection limit (LD)=4.0×10^{-9} g ml^{-1}) [2], HPLC-MS (LD=5.0×10^{-10} g ml^{-1}) [3], column-switching high performance liquid chromatography with fluorescence detection (LD=8.0×10^{-10} g ml^{-1}) [4], column-switching liquid chromatography (LD=1.5×10^{-9} g ml^{-1}) [5], flow-injection chemiluminescence method based on enhancement of the luminol–permanganate reaction (LD=1.7×10^{-10} g ml^{-1}) [6], chemiluminescence method based on potassium ferricyanide oxidation sensitized by rhodamine 6G (LD=6.7×10^{-9} g ml^{-1}) [7], GC–MS (LD=5.0×10^{-10} g g^{-1}) [8] and so

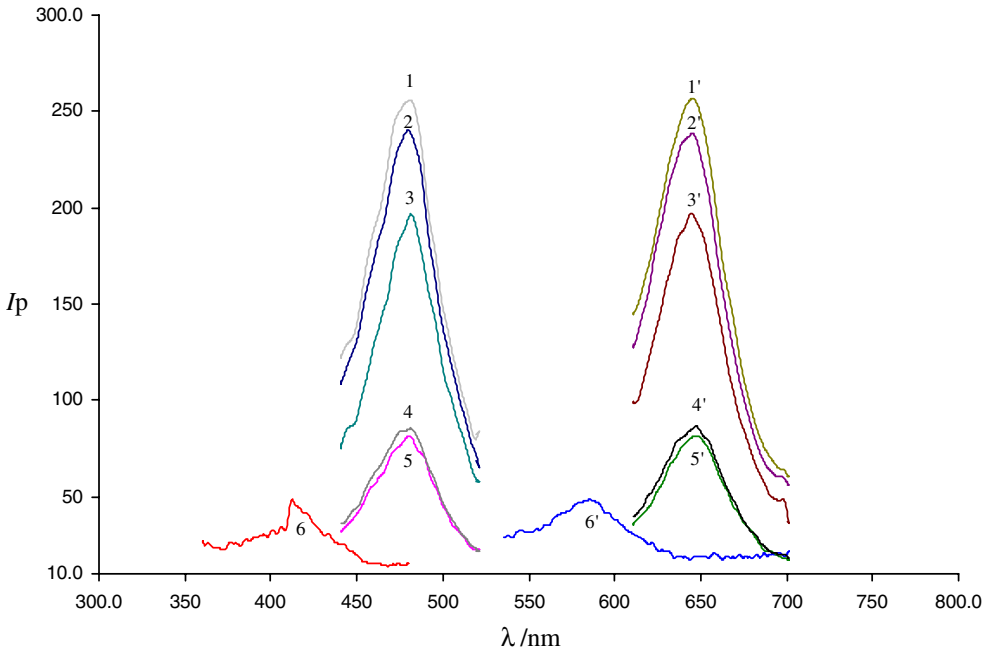


Fig. 1 The phosphorescence spectra of the Rh 6G–$NaIO_4$–buffer solution–TBS system. 1.1′ 1.50 ml Rh 6G, 2.2′ 1.1′+ 2.00 ml buffer solution, 3.3′ 5.5′+130.00 ng TBS, 4.4′ 5.5′+ 0.65 ng TBS, 5.5′ 2.2′+1.50 ml $NaIO_4$, 6.6′ Paper

on, each of these methods has its disadvantage: the sensitivity range of *flow sensor method* is not wide; though HPLC is simple, it is limited for its use of current differential refraction detector, whose sensitivity is low; the sensitivity of chemiluminescence method is not high; GC has to transform the sample into volatile ramification and the operation is complex, it is not suitable for the determination of TBS in medicament.

Our research revealed that Rh 6G could emit strong and stable RTP on filter paper substrate. $NaIO_4$ can oxidize Rh 6G which makes the RTP signal quench. TBS can inhibit $NaIO_4$ from oxidizing Rh 6G which makes the RTP signal enhance sharply. The content of TBS is linear to ΔIp of the system. Based on the facts above, a new $NaIO_4$ oxidize Rh 6G to inhibit SS-RTP for the determination of trace TBS has been established. The LD is 2.6 fg $spot^{-1}$ (corresponding concentration: 6.5×10^{-12} g ml^{-1}) which indicated high sensitivity. This method has been applied to determination of trace TBS in the practical samples with satisfactory results. $NaIO_4$ oxidizing Rh 6G to inhibit SS-RTP for the determination of trace TBS has been rarely reported yet.

Experimental

Apparatus and reagents

Phosphorescent measurements were carried out on a Perkin-Elmer LS-55 luminescence spectrometer with a front–surface attachment (Norwalk, CT 06859–0243, USA). The instrument's main parameters are as following: delay time: 0.1 ms; gate time: 2.0 ms; cycle time: 20 ms; flash count: 1; Ex slit: 10.0 nm, Em slit: 10.0 nm; scan speed: 1,500 nm min^{-1}. pHS-3B precision acidometer; AE240 electron analytical balance (Mettler Toledo instruments company); a 0.5 μl flat head micro-injector (±0.01 μl, Shanghai Medical Laser Instrument Plant) was used to introduce solution.

TBS (China pharmaceutical biology preparation testing centre) working solutions: 0.65 μg ml^{-1} TBS stock solution was diluted to 0.65, 6.50, 65.00 ng ml^{-1}; 1.0×10^{-4} mol l^{-1} Rh 6G solution; 0.50% (*w*/*v*) $NaIO_4$ solution; $KHC_8H_4O_4$-HCl buffer solution (pH=3.50); 1.0 mol l^{-1} I^- solution. All the reagents are A.R. grade except that TBS is primary standard regent. The water used was prepared by thrice quartz sub-boiling distillation.

Table 1 Optimization of the concentration and volume of reagents

Reagents	Concentrations and volumes	The ΔIp in Rh 6G- buffer solution-$NaIO_4$- TBS system	Optimal
Rh 6G (mol l^{-1}) (ml)	10^{-3},10^{-4},10^{-5},10^{-6}	4.1, 17.0, 2.7, 1.2	10^{-4} mol l^{-1}
	0.50,1.00,1.50,2.00,2.50	5.6,12.7, 17.4,11.8,7.3	1.50 ml
$NaIO_4$ (%) (ml)	0.05,0.10,0.50,1.00,3.00	5.0,9.2,17.7,4.5,2.1	0.50%
Buffer	0.50,1.00,1.50,2.00,2.50	7.2,14.1,18.1,10.4,6.0	1.50 ml
Solution (ml)	0.50,1.00,1.50,2.00,2.50,3.00	0.4,0.8,11.5,18.0,7.7,2.5	2.00 ml

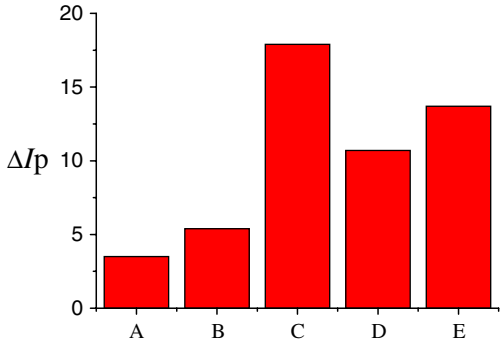


Fig. 2 Effects of luminescence substrates on ΔIp for the reaction system

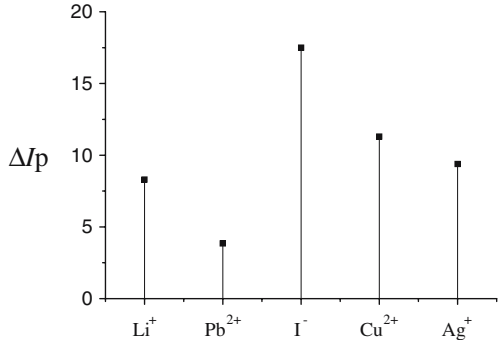


Fig. 4 Effects of heavy atoms on ΔIp for the reaction system

Filter paper was purchased from Xinhua Paper Corporation (HangZhou, China); Polyamide membrane (PAM), acetic acid cellulose membrane (ACM) and nitric acid cellulose membrane (NCM) were purchased from Luqiaosijia Biochemical Plastic Plant. The paper sheets were precut into wafers (Φ=1.5 cm) and a ring indentation was made at the center of the strip with a standard pinhole plotter (φ=4.0 mm) for used.

Experimental methods

To 25 ml colorimetric tube, a certain amount of TBS working solution, 1.50 ml Rh 6G, 2.00 ml buffer solution and 1.50 ml $NaIO_4$ were added, mixed homogeneously, and then diluted to 25 ml with water. The colorimetric tube was heated at 70 °C for 10 min, and then cooled by flowing water for 5 min. The paper prepared was immersed in 1.0 mol l^{-1} KI solution for 10 s and then dried at 90±1 °C for 2 min. A certain amount of test solution was suspended onto the center by a 0.5 μl flat head micro-injector and then the paper was dried at 90±1 °C for 2 min. At the same time, a reagent blank was prepared. The phosphorescence intensity of test solution (Ip_2) and reagent blank (Ip_1) are directly measured at $\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 481/644 nm. Then ΔIp (=Ip_2–Ip_1) was calculated.

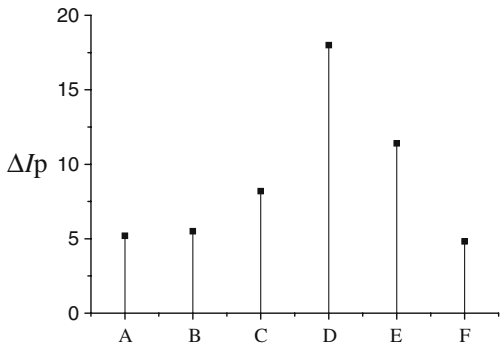


Fig. 3 Effects of oxidants on ΔIp for the reaction system

Results and discussion

Phosphorescence spectra

The phosphorescence spectra of Rh 6G-$NaIO_4$-buffer solution-TBS system were scanned by experimental method (Fig. 1). Results showed that with 1.00 mol l^{-1} I^- as ion perturber, after heated at 70 °C for 10 min, Rh 6G could emit strong and stable RTP ($\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 479.0/644.3 nm, Ip=238.8) on the filter paper. $NaIO_4$ can oxidize Rh 6G to quench the RTP signal ($\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 479.7/647.9 nm, Ip= 81.7). TBS can inhibit $NaIO_4$ from oxidizing Rh 6G, which results in the sharp enhancement of the RTP signal of Rh 6G ($\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 480.6/643.9 nm, Ip=197.2), ΔIp=115.5, $\lambda_{ex}^{max}/\lambda_{em}^{max}$ remained unchanged. So 481/644 nm was chosen as the working wavelength.

Optimum measurement condition

Optimization of the concentration and volume of reagents

For the system containing 0.312 pg TBS $spot^{-1}$, the effects of concentration or volume on ΔIp were studied (Table 1), respectively. Results showed that when the system

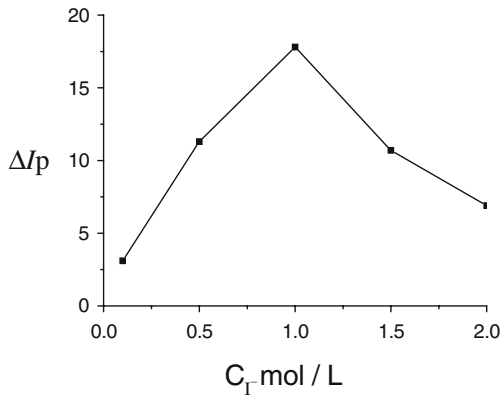


Fig. 5 Effect of the concentration of I^- on ΔIp for the reaction system

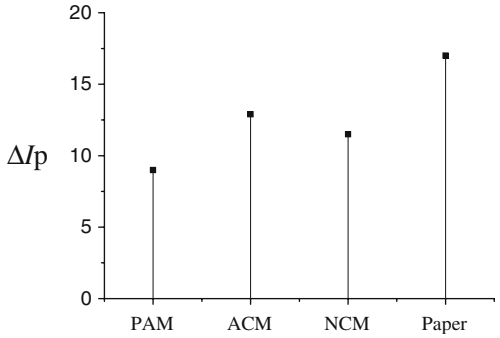


Fig. 6 Effects of substrates on ΔIp for the reaction system

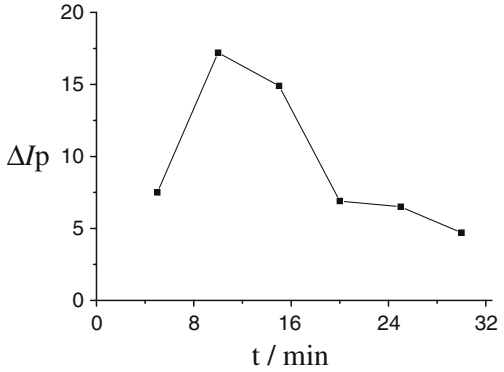


Fig. 8 Effect of time on ΔIp for reaction system

contained: 1.50 ml of 1.0×10^{-4} mol l^{-1} Rh 6G, 1.50 ml of 0.50% (*w*/*v*) $NaIO_4$ and 2.00 ml of buffer solution, ΔIp reached the maximum and remained stable.

Selecting luminescence substrate

For the system containing 0.312 pg TBS $spot^{-1}$, the effects of 0.50 ml of 1.0×10^{-4} mol l^{-1} eosin Y (A), acriflavine (B), Rh 6G (C), calcein (D) and orange yellow G (E) on ΔIp were studied (Fig. 2), respectively. The results showed that when Rh 6G was chosen, ΔIp reached the maximum and remained stable.

Selecting oxidant

For the system containing 0.312 pg TBS $spot^{-1}$, the effects of 1.50 ml of 1.00% (W/V) H_2O_2 (A), KIO_4 (B), $(NH_4)_2S_2O_8$ (C), $NaIO_4$ (D), $KClO_3$ (E) and $KBrO_3$ (F) were studied (Fig. 3), respectively. Results showed that when $NaIO_4$ was chosen, ΔIp reached the maximum and remained stable.

Ion perturber

For the system containing 0.312 pg TBS $spot^{-1}$, the effects of 1.00 mol l^{-1} ion perturbers such as Li^+, Pb^{2+}, I^-, Cu^{2+} and Ag^+ on the system were examined (Fig. 4), respectively. Results showed that ΔIp of I^- system was the highest, so I^- was chosen as ion perturber. At the same time, the effects of different concentrations of I^- on ΔIp of the system were examined (Fig. 5). Results showed that ΔIp of 1.0 mol l^{-1} I^- was the highest, so 1.0 mol l^{-1} I^- was chosen.

Selecting solid substrate

For the system containing 0.312 pg TBS $spot^{-1}$, the effects of different substrates, such as PAM, ACM, NCM and paper on ΔIp were examined (Fig. 6). Results showed that when paper was chosen, the ΔIp reached the highest (Fig. 7).

Temperature and time for reaction

For the system containing 0.312 pg TBS $spot^{-1}$, the effects of reaction time and temperature on ΔIp were examined (Fig. 8–9), respectively. When the reaction temperature was 70 °C, and the time was 10 min, ΔI_P reached the maximum and remained stable.

Acidity for reaction

For the system containing 0.312 pg TBS $spot^{-1}$, the effects of pH=3.01, 3.50, 5.23, 11.50 and 12.05 on ΔIp were

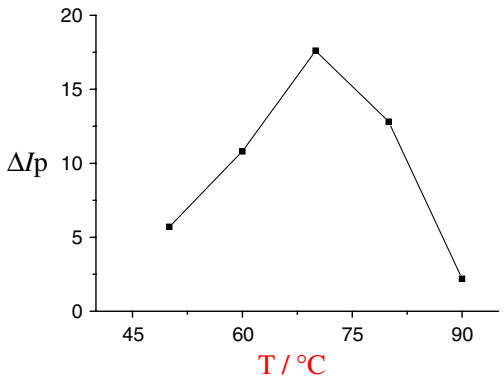


Fig. 7 Effect of temperature on ΔIp for reaction system

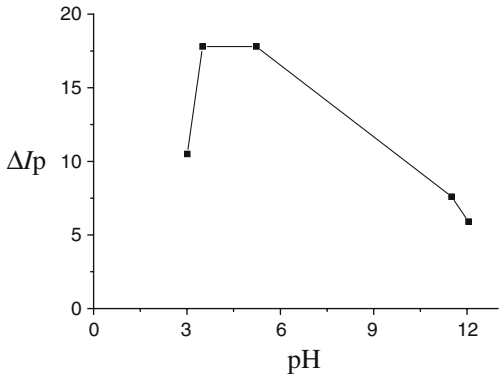


Fig. 9 Effect of acidity on ΔIp for reaction system

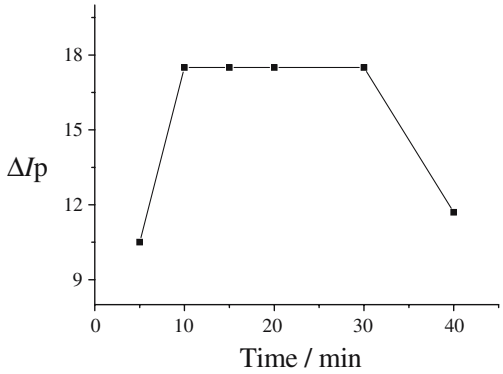


Fig. 10 Stability of the reaction system

examined (Fig. 9), respectively. When pH values are below 3.50 and above 11.50, ΔIp of the system was less, while pH values were among 3.50–5.23, ΔI_P reached the maximum and remained stable. Buffer solution, 2.00 ml, was used to control the acidity of the solution and the value of pH was 3.50.

Stability of the reaction system

For the system containing 0.312 pg TBS spot^{-1}, the stability of the reaction system was studied under the optimum conditions above (Fig. 10). Results showed that ΔIp remained almost unchanged among 10–30 min.

Working curve, linear range and detection limit

The amount of TBS is linear correlated to ΔIp of the system among 0.0104–2.08 pg spot^{-1} (corresponding concentration: 0.026–5.2 ng ml^{-1}, with a sample volume of 0.4 μl) (Fig. 11), and the regression equation of working curve can be expressed ΔIp=2.040+54.54 m_{TBS} (pg spot^{-1}), n=6, correlation coefficient is 0.9994. For the samples containing 0.0104 and 2.08 pg spot^{-1} TBS, the relative standard deviation (RSD) are 3.8% and 2.3% (n=8), respectively, indicating good precision. The detection limit calculated by the method of 3Sb/K is 2.6 fg spot^{-1} (corresponding concentration of TBS: 6.5×10^{-12} g ml^{-1}, n=11), indicating high sensitivity.

Interference experiment

For the system of 0.312 pg TBS spot^{-1}, the allowed concentration (multiple) of coexistence ions (Er=±5%) are as following: NO_3^-, K^+, Na^+, Ca^{2+}, HCO_3^-, CO_3^{2-}, glucose, galactose, lactose and starch (1.0×10^4), Zn^{2+}, Al^{3+}, Mg^{2+}. Cl^-, Br^-, PO_4^{3-}, SO_4^{2-}, Fe^{2+}, Fe^{3+} and dextrine (2.0×10^3), vitamine C, citric acid and sucrose (100), indicating good selectivity of this method.

Lifetime of phosphorescence

The RTP lifetime (t) obtained by phosphorescence attenuation curve (delay time: 0.1 ms; gate time: 2.0 ms) of sample containing 0.312 pg TBS spot^{-1} is 11.9 ms [9]. According to the method in literature, the regression equation of the attenuation curve can be expressed as ln Ip=3.316–0.0842 t (r=−0.9967).

Analysis of samples

TBS tablets were purchased from the local hospital. The average tablet weight was calculated from the weight of 20 tablets. They were grounded to fine powder. Weighed 0.2 g sample (±0.01 mg, 2.5 mg of TBS per tablet) accurately to the beaker and dissolved in 100 ml water, 1.00 ml of the solution was diluted to 1000 ml as working solution.

According to the above method, the content of TBS in 1.00 ml of the solution was determined and the results were compared with chemiluminescence method (CL) [7]. Meanwhile, a standard addition recovery experiment was carried out; the results were listed in Table 2.

The reaction mechanism

Under the condition of 70 °C and 10 min, with 1.00 mol L^{-1} I^- as ion perturber, TBS can emit RTP ($\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 453.1/622.1 nm, Ip=77.1) on the paper solid substrate, but in the presence of $NaIO_4$ RTP signal of TBS quenched (Fig. 12), which indicated TBS could be oxidized by $NaIO_4$.

Rh 6G can emit RTP (Fig. 1, Curve 2.2′) on the paper solid substrate, and RTP signal of Rh 6G changed minutely in the presence of TBS (Fig. 13), which indicated that TBS could hardly react with Rh 6G.

However, the RTP signal of Rh 6G quenched intensively in the Rh 6G-$NaIO_4$-buffer solution system (Fig. 1, Curve 5.5′), maybe Rh 6G was oxidized by $NaIO_4$ to non-

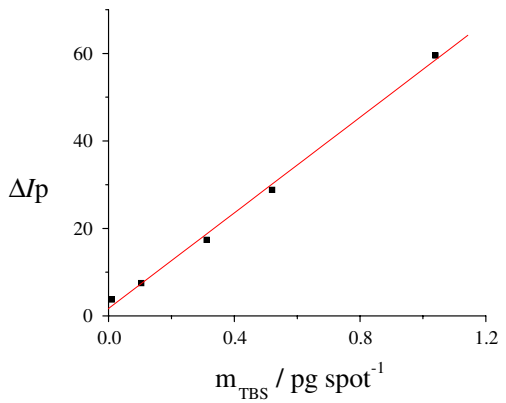
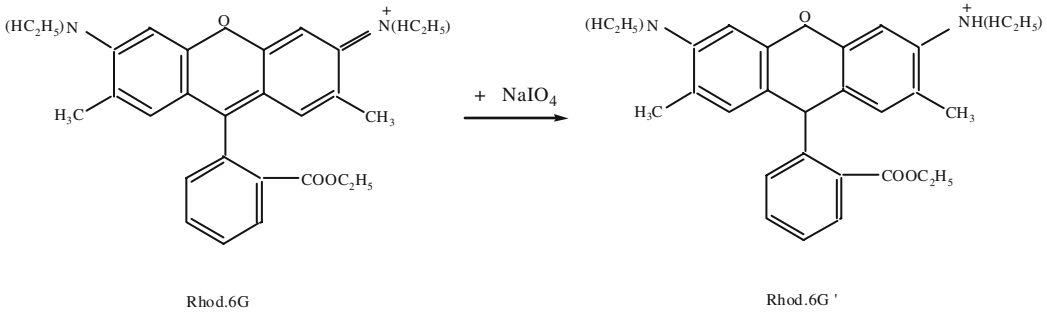


Fig. 11 Working curve

Table 2 Analysis results of TBS in samples

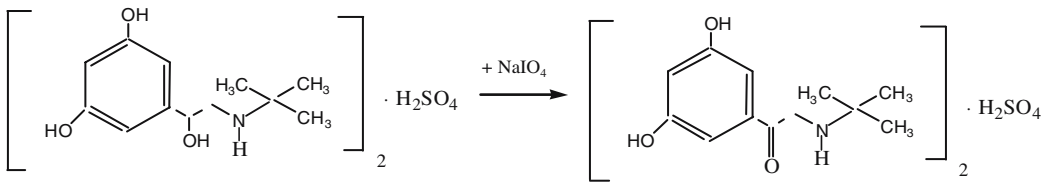
Sample number	Found (mg g^{-1})	RSD (%)	Added (mg g^{-1})	Obtained (mg g^{-1})	Recovery (%)	CL (mg g^{-1})	Relative error (%)
1	13.4	2.2	1.35	1.34	99.2	14.0	−4.3
2	13.6	1.3	1.35	1.36	100.4	14.0	−2.9
3	13.5	1.1	1.35	1.38	102.0	14.0	−3.6

phosphorescence complex, and the oxidation reaction can be expressed as follows:



Rhod.6G Rhod.6G '

The oxidization-reduction reaction expression took place between TBS and $NaIO_4$ when TBS was added. The reaction can be expressed as follows:



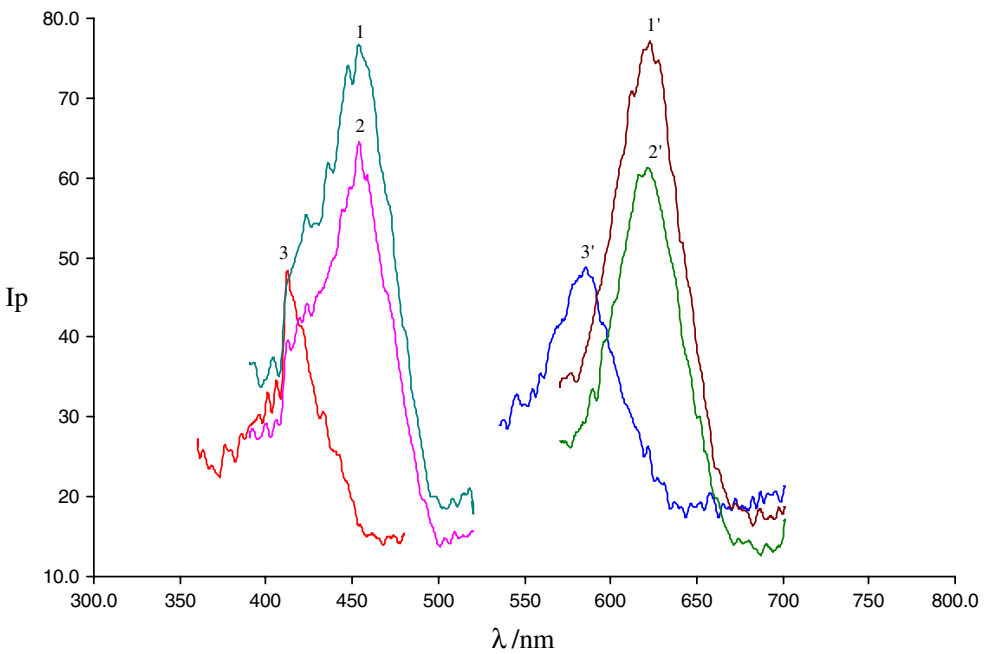


Fig. 12 The phosphorescence spectra of the TBS–buffer solution–$NaIO_4$ system 1.1′ 2.00 ml buffer solution+130 ng TBS, 2.2′ 1.1′+1.50 ml $NaIO_4$, 3.3′ Paper

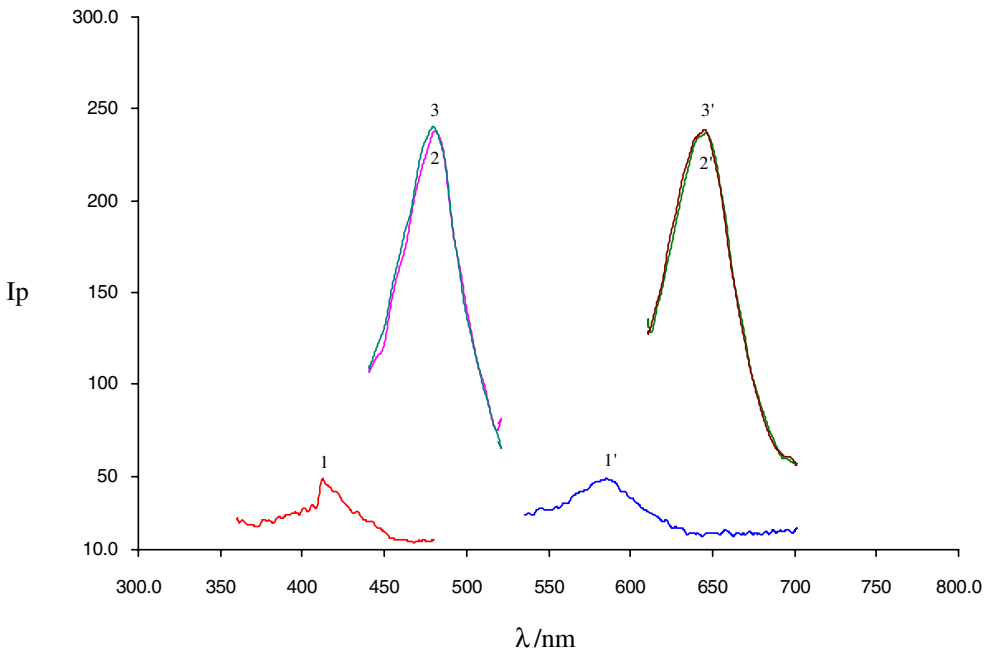


Fig. 13 The phosphorescence spectra of the Rh 6G–buffer solution–TBS system, 1.1′ Paper, 2.2′ 3.3′+130 ng TBS, 3.3′ 1.50 ml Rh 6G+2.00 ml buffer solution

The above reaction inhibited $NaIO_4$ from oxidizing Rh 6G, which caused the phosphorescence signal of Rh 6G to enhance sharply, and ΔIp of the system was linear correlation to the content of TBS. According to the facts above, trace TBS can be determined by inhibition SS-RTP based on $NaIO_4$ oxidizing Rh 6G.

Conclusion

A new SS-RTP for the determination of trace TBS based on the inhibition effect of TBS on $NaIO_4$ oxidizing Rh 6G has been established. This method is sensitive and accurate, with a detection limit of 2.6 fg spot^{-1} (corresponding concentration: 6.5×10^{-12} g ml^{-1}). The method has been applied to the determination of trace TBS in the medicament, which has provided a new method for the medicament analysis. It has also driven the research progress of the detection technology of the exhilarant.

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References

1. Editorial Committee of Pharmacopoeia of People's Republic of China (2000) The Pharmacopoeia of People's Republic of China, 2nd edn. Chemical Industry, Beijing, p 890
2. He D et al (2006) Talanta 69(5):1215
3. Yamini Y et al (2006) J Chro A 1124(1–2):57
4. Qin YP et al (2004) Chin J Anal Chem 32(9):1216
5. Zhang Y et al (2004) J Chro B 805(2):211
6. Wang ZP et al (2004) Anal Bioanal Chem 378(30):834
7. Lv Y et al (2003) J Pharm Biomed Anal 32(3):555
8. Wu PG et al (2005) J Hygiene Res 34(3):365
9. Wei YS et al (1998) Chin J Anal Chem 26(5):515