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Highly Sensitive Detection of Residual Chlorpromazine Hydrochloride with Solid Substrate Room Temperature Phosphorimetry

Jia-Ming Liu · Li-ping Lin · Xin-Xing Wang · Xuan Lin · Ping Zhou · Shao-Qin Lin · Zhi-Yong Zheng

Abstract Under the condition of 60 °C and 20 min at pH 6.12, chlorpromazine hydrochloride (CPZ) could react with fluorescein isothiocyanate (FITC) to produce FITC-CPZ, which increased the π-electron density (δ) of carbon atom in FITC conjugated system and the room temperature phosphorescence (RTP) intensity of FITC. Thus, a new solid substrate room temperature phosphorimetry (SSRTP) for the determination of residual CPZ was established. The regression equation of working curve was ΔI_p=4.254+ 7.906 m_{CPZ} (ag spot^{-1}) with the correlation coefficient (r) of 0.9990 in the range of 0.036–9.6 ag spot^{-1} (corresponding concentration: 0.090–24 fg ml^{-1}, sample volume: 0.40 μl spot^{-1}), and the detection limit (LD) was 0.018 ag spot^{-1} (corresponding concentration: 4.5×10^{-17} g ml^{-1}). This method with wide linear range and high sensitivity was not only used to diagnose human disease based on the correlation between the residual quantity and lethal dose of CPZ in human serum, but also used to determine residual CPZ in biological samples with the results consisting with those obtained by gas chromatography (GC), showing good accuracy. The constituent of FITC-CPZ was analyzed by GC-MS (mass spectrometry) and the

reaction mechanism of SSRTP for the determination of trace CPZ was also discussed.

Keywords Chlorpromazine hydrochloride · Fluorescein isothiocyanate · π-electron effect · Solid substrate room temperature phosphorimetry · Residue analysis

Introduction

CPZ not only has protective effect on brain injury in rats [1], but also can be used to determine some anionic surfactants [2] and metal ions [3] in environmental water samples, showing great promise in a variety of applications in medicine, environmental science and analytical chemistry. However, excess CPZ has inhibiting effect on neural system, respirometric system and circulative system. Generally, oral lethal dose is 15–150 mg CPZ kg^{-1}, and lethal blood concentration is 5–10 mg CPZ L^{-1} [4]. Therefore, the determination of residual CPZ in human serum is of great significance in the clinical detection.

In this paper, the RTP spectra of FITC, CPZ and FITC-CPZ were studied. It has been found that the RTP signal of FITC in the FITC-CPZ sharply enhanced because the π-electron density of carbon atom in the FITC-CPZ conjugated system increased. Hereby, a novel SSRTP for the determination of trace CPZ was developed. This method with simple and easy operation is more suitable for the residue analysis of CPZ in human serum and urine due to the higher sensitivity than those in references [3–9], wider linear range than those in references [3–10] and better selectivity than that in Ref. [3]. Besides, the SSRTP for the determination of trace CPZ based on the π-electron effect of carbon atom in FITC conjugated system has been rarely reported. The study not only developed a new method for the determination of

J.-M. Liu (✉) · L.-p. Lin · X.-X. Wang · X. Lin · P. Zhou
Department of Chemistry and Environmental Science,
Zhangzhou Normal College,
Zhangzhou 363000, People's Republic of China
e-mail: zzsyliujiaming@163.com

S.-Q. Lin
Department of Biochemistry, Fujian Education College,
Fuzhou 350001, China

Z.-Y. Zheng
Department of Food and Biological Engineering,
Zhangzhou Institute of Technology,
Zhangzhou 363000, People's Republic of China

residual CPZ, but also provided a new technique for the CPZ clinical detection, which promoted the progress in study of trace drugs analysis.

Experimental

Apparatus and Reagents

Phosphorescence measurements were carried out on an LS-55 fluorescence spectrophotometer with a solid surface analysis apparatus (Perkin-Elmer Corporation USA). The instrument's main parameters are as follows: delay time 0.1 ms, gate time 2.0 ms, cycle 20 ms, flash count 1, Ex slit 10.0 nm, Em slit 10 nm, speed 1500 nm min^{-1}. AE240 electronic analytical balance (Mettler-Toledo Instruments Company Limited), pHS-3B acidimeter and a 0.50-μl flat head micro-injector (Shanghai Medical Laser Instrument Plant) were used.

CPZ working solution: 0.0100 g CPZ standard reagent was weighed accurately (Beijing institute for the control of pharmaceutical and biological products), dissolved in water and diluted to 100 mL with water to prepare stock solution of 100.00 μg mL^{-1}, and then diluted it to 100.00, 10.00 and 1.00 (fg mL^{-1}) gradually with water before use. FITC solution: 1.0×10^{-3} mol L^{-1} FITC stock solution was prepared with ethanol, and diluted to 1.0×10^{-4} mol L^{-1} with 1.0×10^{-4} mol L^{-1} HAc before use. 1.0×10^{-5} mol L^{-1} NaOH solution and 1.0 mol L^{-1} Pb(Ac)$_2$ solution (dissolved with 2.0 mol L^{-1} HAc solution) were used. All reagents were analytical reagents except that CPZ was standard reagent. The water used was prepared by thrice sub-boiling distillation.

Filter paper, polyamide membrane (PAM), acetyl cellulose membrane (ACM) and nitrocellulose membrane (NCM) were purchased from Luqiaosijia Biochemical Plastic Plant. They were cut into wafers (Diameter is 15 mm.) and a ring indentation (Diameter is 4.0 mm.) was made at the center of each sheet with a standard pinhole plotter for use.

Experimental Method

Proper 100.00 fg CPZ mL^{-1}, 1.50 mL of 1.0×10^{-4} mol L^{-1} FITC and 1.00 mL of 1×10^{-5} mol L^{-1} NaOH were added into a 25-mL colorimetric tube and diluted with water, and finally mixed homogeneously. The colorimetric tube was kept at 60 °C for 20 min, and then cooled by flowing water for 5 min. The PAM was immersed in 1.0 mol L^{-1} Pb(Ac)$_2$ solution for 10 s, and dried at 90±1 °C for 2 min. A 0.40 μL of test solution was suspended onto the center of the PAM wafer with a 0.50-μl flat head micro-injector, and then dried at 90±1 °C for 2 min. Simultaneously, a reagent blank experiment was also conducted. The phosphorescent intensity was measured directly at 478/642 nm ($\lambda_{ex}^{max}/\lambda_{em}^{max}$). The emission phosphorescent intensity of both test solution FITC-CPZ (I_{p1}) and the blank reagent FITC (I_{p2}) were recorded. Each sample was measured for six times. Then, the ΔI_p (= $I_{p1} - I_{p2}$) of system was calculated.

Preparation of Analytical Sample

The serum and urine of the psychotic patients who took CPZ tablet 100 mg per day were measured at various time points after 12 days, respectively. Human serum was treated with the method described in the Ref. [11]. Briefly, 3.00 mL of the venous blood was phlebotomized from the patients who took CPZ, and added heparin anticoagulant, then centrifuged at the speed of 2000 r min^{-1} for 5 min. After that, 1.00 mL upper serum was taken, mixing with 10 μL of 2.50 mg mL^{-1} ascorbic acid, diluted to 10 mL with water, and finally placed in the refrigerator for use. Took 1.00 mL of serum stock solution and diluted it to 10^6 times with water for testing.

Fig. 1 RTP spectra for the system (Curves 1–5 are the excitation spectra, and curves 1′-5′ are the emission spectra. 1.1′, 2.2′, 3.3′, 4.4′ and 5.5′ are number of the excitation spectra and the emission spectra. Two sets of data corresponding 1′-5′ are emission wavelength and intensity of RTP, respectively.)
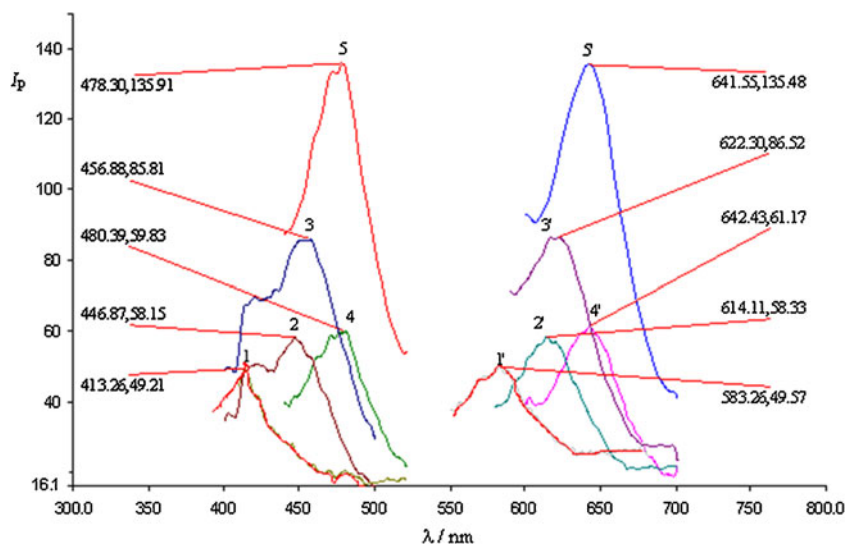
Table 1 RTP characteristics of FITC-CPZ (RSD (relative standard deviation) was obtained by determining the I_p of the system for 6 times. $\Delta I_p = I_{p1} - I_{p2}$)

The FITC-CPZ system	λ_{ex}^{max}	λ_{em}^{max}	I_p	RSD%	ΔI_p	pH
1.1′ PAM	413.3	583.3	49.6	4.3		
2.2′ 1.50 mL FITC-HAc	446.9	614.1	58.3	3.7		1.04
3.3′ 1.50 mL FITC + 1.00 mL NaOH	456.9	622.3	86.5	3.4		6.12
		641.6	54.4 (I_{p2})	4.1		
4.4′ 3.3′+3.0 fg CPZ	480.4	642.4	58.3 (I_{p1})	3.9	3.9	
5.5′ 3.3′+600.0 fg CPZ	478.3	641.6	135.5 (I_{p1})	3.2	78.9	

According to the method mentioned in Ref. [9], 10.00 mL of human urine was taken and 0.10 mL of 5% NaOH, 1.0 g NaCl and 5.0 mL ethyl acetate were added, and mixed for 1 min and stood for 10 min. 5% HCl was used to adjust the pH of the solution to 6.12 and then it was diluted to 100 mL with water for use. Took the 1.00 mL of human urine stock solution and diluted it to 10^9 times with water for testing.

GC-MS Analysis of FITC-CPZ

3.6 fg CPZ, 1.50 mL FITC and 1.00 mL NaOH were added into a 25-mL colorimetric tube and diluted with water, and finally mixed homogeneously. The mixed solution was kept at 60 °C for 20 min, transferred to the rotating evaporator, added 10 mL ethyl acetate, and then extracted for twice. At last, the extracted supernatants carefully evaporated just to dryness with a vacuum evaporator. The residue was dissloved with 2.00 mL acetonitrile by ultrasonic, then 4.00 mL n-hexane was added. Then, the solution was centrifugated at the speed of 1000 r min^{-1} for 5 min and n-hexane layer was abandoned and the extracted solution was obtained for use. C-18 pillar was washed by 2.00 mL acetonitrile. Then, the extracted solution was passed into C-18 pillar. The passed solution was deserted and C-18 pillar was washed again by 1.00 mL water. 2.00 mL of 2% ammoniated ethyl acetate were sequently passed through C-18 pillar

Table 2 Measurement conditions (A, B and C are fluorescein, tetrabromofluorescein and tetrachlorotetraiodo fluorescein, respectively)

Effect factors	Factor variables	The ΔI_p of the system	Optimal
Luminescence materials RSD%	A, B, C, FITC	9.1, 11.3, 13.1, 16.6 4.7, 4.0, 3.6, 3.3	FITC
FITC (mol L^{-1}) RSD%	10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}	11.2, 16.3, 12.8, 10.4, 9.6 3.9, 3.4, 3.7, 4.4, 4.6	1.0×10^{-4} mol L^{-1}
FITC (mL) RSD%	0.50, 1.00, 1.50, 2.00, 2.50, 3.00	8.9, 12.8, 16.5, 13.6, 10.9, 8.3 4.8, 3.6, 3.2, 3.5, 4.4, 4.9	1.50 mL
Pb^{2+} (mol L^{-1}) RSD%	0.050, 0.10, 0.30, 0.50, 1.0, 1.2	8.0, 10.9, 12.7, 14.6, 16.4, 14.2 5.0, 4.8, 3.6, 3.4, 3.1, 3.5	1.0 mol L^{-1}
pH of the reaction system RSD %	3.03, 4.14, 5.07, 6.12, 6.62, 7.01	9.7, 12.5, 15.6, 16.4, 16.2, 16.3 4.6, 3.7, 3.4, 3.2, 3.3, 3.1	6.12
Reaction temperature (°C) RSD %	50, 55, 60, 65, 70	9.8, 13.2, 16.2, 15.3, 13.9 4.5, 3.4, 3.0, 3.3, 3.4	60 °C
Reaction time (min) RSD%	5, 10, 15, 20, 25	14.6, 15.5, 16.1, 16.5, 16.3 3.4, 3.2, 3.0, 3.3, 3.1	20 min
Drying temperature (°C) RSD%	80, 85, 90, 95	10.6, 15.4, 16.2, 15.8 4.7, 3.4, 3.1, 3.3	90 °C
Drying time (min) RSD%	0.5, 1.0, 1.5, 2.0, 2.5, 3.0	9.7, 12.4, 15.3, 16.5, 16.4, 16.3 4.6, 3.5, 3.3, 3.0, 3.2, 3.1	2.0 min
Passing drying N$_2$ (min) RSD%	10, 20, 30, 40, 50	16.0, 16.4, 16.1, 16.3, 16.2 3.3, 3.1, 3.4, 3.0, 3.2	10 min
Not passing N$_2$ (min) RSD%	10, 20, 30, 40, 50	15.4, 13.2, 12.4, 10.1, 8.3 3.4, 3.6, 3.7, 4.8, 5.0	
Standing time (min) RSD%	10, 20, 30, 40, 50, 60	16.3, 16.4, 16.3, 16.4, 16.4, 14.2 3.1, 3.0, 3.3, 3.2, 3.1, 3.4,	10–50 min
Solid substrate RSD%	PAM, NCM, ACM, paper	16.3, 10.6, 11.3, 9.2 3.0, 4.6, 4.1, 4.7	PAM
Ion perturber RSD%	Li$^+$, Cu^{2+} Ag$^+$, Pb^{2+}, I$^-$	8.6, 11.9, 13.3, 16.3, 10.8 4.8, 4.0, 3.5, 3.2, 4.5	Pb^{2+}

Table 3 Linear range, the regression equation, r, RSD, LD and LOQ (RSD (%) were calculated from the determined results for the samples containing 0.048 and 9.6 ag CPZ spot^{-1} for 7 times, respectively. The LD is calculated by 3Sb/k, thereinto, 3 Sb/k referred to the quotient between triple of the blank reagent's standard deviation and the slope of the working curve; Sb referred to the standard deviation of 11 parallel analysis of the blank reagent; Sb=0.048. And LOQ is calculated by 10 Sb/k.

Method	Linear range (g mL^{-1})	r	RSD (%)	LD (g mL^{-1})	LOQ (g mL^{-1})
This method	0.036–9.6 (ag spot^{-1}) 9.0×10^{-17}–24×10^{-15}	0.9990	3.3–4.0	0.018 (ag spot^{-1}) 4.5×10^{-17}	0.059 (ag spot^{-1}) 1.5×10^{-16}
Spectrofluorimetry [3]	0–2.0×10^{-11}	0.9908	2.5	1.0×10^{-8}	3.3×10^{-8}
Spectrophotometry [6]	8.3×10^{-3}–6.7×10^{-2}	0.9994	1.38	2.3×10^{-6}	7.6×10^{-6}
Chemiluminescence method [7]	5.0×10^{-8}–1.0×10^{-5}	0.99	2.6	6.0×10^{-9}	2.0×10^{-8}
Gas chromatography-mass Spectroscopy [8]	1×10^{-9}–1.0×10^{-7} (g g^{-1})	0.9995	5.9–9.4	1.0×10^{-9} (g g^{-1})	3.3×10^{-9} (g g^{-1})
Gas chromatography [9]	0–1.0×10^{-4}	0.9993	3.0–4.2	5.0×10^{-8}	
High performance liquid chromatography [10]	1.0×10^{-8}–1.6×10^{-7}	0.997	3.0–4.3		1.6×10^{-7}

and only the final eluent was collected. The eluent was evaporated to dryness by nitrogen blowing apparatus, 0.20 mL ethyl acetate was added, mixed homogeneously with ultrasonic and finally introduced sample into GC-MS.

Results and Discussion

RTP Spectra

The RTP spectra of the FITC-CPZ system were scanned (Fig. 1). Results show that FITC could emit strong and stable RTP signal on the PAM in the presence of perturber Pb^{2+} at 60 °C for 20 min (Table 1). When pH values in the system were 1.04 and 6.12, the $\lambda_{ex}^{max}/\lambda_{em}^{max}$ of the FITC were 446.9/614.1 nm and 456.9/622.3 nm, and the I_p values of the system were 58.3 (Fig. 1, curve 2.2′) and 86.5 (Fig. 1, curve 3.3′), respectively. The remarkable differences in the RTP characteristics might be closely correlated with the pH of the system and the existence form of FITC [12]. When pH of the system was 6.12, 600.0 fg CPZ could cause the RTP signal of FITC to sharply enhance ($\lambda_{ex}^{max}/\lambda_{em}^{max} = 478.3/641.6\,nm$, $I_p=135.5$, $\Delta I_p=78.9$, Fig. 1, curve 5.5′) and λ_{em}^{max} red shifted for 19.3 nm, which might be due to the formation of FITC-CPZ compound containing –CS–NH– between the reaction of –NH$_2$ of CPZ and the –NCS of FITC.

Optimum Measurement Conditions

For the system containing 1.4 ag CPZ spot^{-1}, the effects of the luminescence materials, concentration and volume of reagents, reaction acidity, reaction temperature and time, drying temperature and time, oxygen, standing time, solid substrate, the species of ion perturber and concentrations of Pb^{2+} on the ΔI_p of the system were tested, respectively (Table 2). Results show that the ΔI_p of the system reached the maximum and corresponding RSDs (%) for the ΔI_p of the system were within ±5% when 1.50 mL of 1.0×10^{-4} mol L^{-1} FITC and
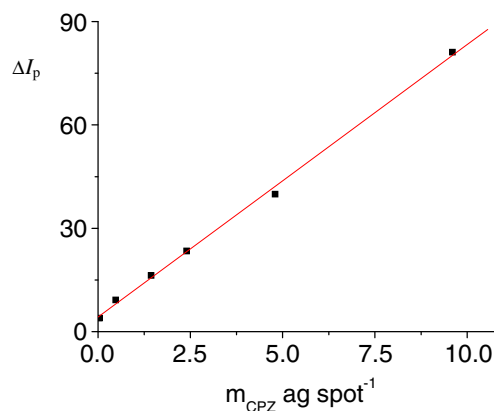
Fig. 2 The working curve for the determination of trace CPZ ($\Delta I_p=4.254+7.906\ m_{CPZ}$ (ag spot^{-1}), $n=6$)

1.0 mol L^{-1} Pb^{2+} were used, the pH of reaction system was 6.12, incubated at 60 °C for 20 min, drying temperature was 90 °C and the time was 2.0 min, the time of passing drying N$_2$ was 10 min as well as PAM was used as solid substrate.

From Table 2, we could conclude the following rules:

1. The ΔI_p of system increased in turn by the order of fluorescein, tetrabromofluorescein, tetrachlorotetraiodo fluorescein and FITC. Thereinto, the ΔI_p of system reached the maximum and remained stable when FITC was used. The reason might be that –NCS of FITC could easily react with –NH$_2$ of CPZ to from FITC-CPZ compound.

2. The ΔI_p of the system increased gradually with the increase of Pb^{2+} concentration. We reasoned that the heavy atom effect of Pb^{2+} can greatly increase the transition probability from the singlet state (S$_1$) to triplet state (T$_1$) of the luminescence molecule. However, when the concentration of Pb^{2+} was over 1.0 mol L^{-1}, the ΔI_p of the system declined, indicating that the proper amount of Pb^{2+} would enhance the ΔI_p value of the system, while the superfluous Pb^{2+} only quenched the RTP.

3. The ΔI_p of system enhanced gradually with the increasing pH value of the solution due to the increasing yield of FITC-CPZ. The ΔI_p value of system reached the maximum when the pH value of the solution was 6.12 resulting from the highest yield of FITC-CPZ. The ΔI_p of system declined gradually when the pH value of the solution exceeded 7.0, which might be the result that CPZ gradually hydrolyzed with the increasing pH value and the decreasing yield of FITC-CPZ.

4. The ΔI_p of system gradually increased with the increase of reaction temperature and time, whereas decreased gradually when the temperature was over 60 °C and the time was over 20 min. The reason was that the dissociation of CPZ led to the decline of the FITC-CPZ yield.

5. With the increase of the drying temperature and time, the ΔI_p of the system gradually increased due to the evaporation of the water on solid substrate. The ΔI_p of the system reached the maximum and remained stable when the drying temperature was 90 °C and the time was 2 min. When the drying temperature was over 90 °C and the time was over 2 min, the ΔI_p of the system to gradually decrease owing to the decomposition of FITC-CPZ.

6. The remarkable quenching effects of oxygen and humidity on the ΔI_p of the system could be eliminated in the presence of drying N$_2$. Results show that the ΔI_p of the system almost stayed invariable when drying N$_2$ was passed for 10 min.

7. After being cooled for 5 min with water, the ΔI_p of the system almost stayed invariable within 50 min. When the standing time exceeded 50 min, the solid substrate absorbed the water in the air, causing the quenching of RTP and sharp decrease of the ΔI_p of the system.

Working Curve, Linear Range, Precision and Sensitivity

Linear range, the regression equation of working curve, correlation coefficient (r), RSD, the LD and limit of quantity (LOQ) of this method were compared with those in Refs. [3, 6–10], and the results are listed in Table 3. Fig. 2 shows the working curve of this method.

Concluded from Table 3, this method has lower LD than those in references [3, 6–9], and wider linear range than those in references [3, 6–10], indicating that this method is suitable for trace CPZ detection.

Table 4 Effects of coexistent materials (CI is coexistent ion; CM is coexistent materials; C is allowed maximum concentration of coexistent ions (materials); M referred to the quotient between allowed maximum concentration of coexistent ions (materials) and the concentration of CPZ. β-CD is β-cyclodextrin)

Present method			Ref. [3]		Present method			Ref. [3]	
CI	C (g mL^{-1})	M	C (g mL^{-1})	M	CI or CM	C (g mL^{-1})	M	C (g mL^{-1})	M
Cd^{2+}, Cl$^-$	9.0×10^{-11}	25000	5.0×10^{-4}	1000	Al^{3+}, Cl$^-$	3.6×10^{-12}	1000	4.0×10^{-5}	80
Na$^+$, Cl$^-$	9.0×10^{-11}	25000	5.0×10^{-4}	1000	CO$_3$$^{2-}$, H$^+$	3.6×10^{-12}	1000	1.0×10^{-5}	20
K$^+$, Cl$^-$	9.0×10^{-11}	25000	5.0×10^{-4}	1000	Fe^{3+}, Cl$^-$	$1,8\times10^{-12}$	500	1.0×10^{-6}	2
Cl$^-$, H$^+$	9.0×10^{-11}	25000	5.0×10^{-4}	1000	EDTA	9.0×10^{-11}	25000	5.0×10^{-4}	1000
C$_2$O$_4$$^{2-}$, H$^+$	9.0×10^{-11}	25000	5.0×10^{-4}	1000	Glucose	9.0×10^{-11}	25000	5.0×10^{-4}	1000
Mg^{2+}, Cl$^-$	7.2×10^{-11}	20000	2.5×10^{-4}	500	Sucrose	9.0×10^{-11}	25000	5.0×10^{-4}	1000
Cu^{2+}, Cl$^-$	7.2×10^{-11}	20000	2.5×10^{-4}	500	Amylum	9.0×10^{-11}	25000	4.0×10^{-4}	800
SO$_4$ $^{2-}$, H$^+$	7.2×10^{-11}	20000	1.5×10^{-4}	300	Lactose	7.2×10^{-11}	20000	2.5×10^{-4}	500
NH$_4$$^+$, Cl$^-$	7.2×10^{-11}	20000	1.2×10^{-4}	250	β-CD	7.2×10^{-11}	20000	2.0×10^{-4}	400
Co^{2+}, Cl$^-$	5.4×10^{-11}	15000	1.0×10^{-4}	200	Carbamide	5.4×10^{-11}	15000	1.0×10^{-4}	200
Zn^{2+}, Cl$^-$	5.4×10^{-11}	15000	1.0×10^{-4}	200	Dextrin	5.4×10^{-11}	15000	1.0×10^{-4}	200
Ac$^-$, H$^+$	5.4×10^{-11}	15000	7.0×10^{-5}	140	Vitamin C	3.6×10^{-12}	1000	2.5×10^{-5}	50

Table 5 Sample analysis (Conditions of GC: chromatogram column: DB-1701 (30 m×0.32 mm×0.25 μm) quartz flexible capillary column; injection port temperature was 250 °C, detector (NPD) temperature was 300 °C. The initial temperature was 180 °C and maintained for 1 min. The temperature wa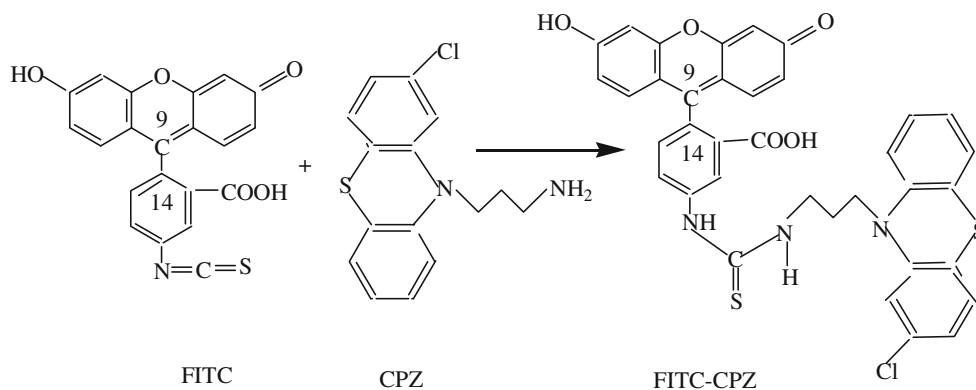s then raised to 240 °C at a rate of 20 °C min^{-1} and maintained for 8 min. Flow rate of N$_2$, H$_2$, air and tail-blowing gas were 3.0 mL min^{-1}, 4.5 mL min^{-1}, 300 mL min^{-1} and 25 mL min^{-1}, respectively. Split ratio was 1: 5. Qualitative and quantitative analysis were operated by the retention time and peak area, respectively)

Sample	Found (μg mL^{-1})	RSD (%)	Added (μg mL^{-1})	Obtained (μg mL^{-1})	Recovery (%)	Found (GC) (μg mL^{-1})	E_r (%) (GC)
Serum A	0.0301	4.5	0.0018	0.00180	100	0.0309	−2.6
Serum B	0.0309	4.3	0.0018	0.00182	101	0.0313	−1.3
Serum C	0.0298	4.7	0.0018	0.00179	99.4	0.0305	−2.3
Serum D	0.0312	4.1	0.0018	0.00181	100.6	0.0307	+ 1.6
Serum E	0.0303	4.6	0.0018	0.00180	100	0.0308	−1.6
Serum F	0.0307	4.2	0.0018	0.00182	101	0.0314	−2.3
Serum G	0.0302	4.5	0.0018	0.00181	100.6	0.0315	−4.1
Serum H	0.0308	4.3	0.0018	0.00183	101.7	0.0307	+ 3.2
Serum I	0.0304	4.6	0.0018	0.00181	100.6	0.0309	−1.6
Serum J	0.0306	4.2	0.0018	0.00182	101	0.0313	−2.2
Average	0.0305	4.4	0.0018	0.00183	100.6.	0.0311	
Urine A	76.5	4.0	7.50	7.49	99.9	76.7	−2.6
Urine B	77.3	3.4	7.50	7.51	100.1	77.4	−1.3
Urine C	76.6	3.8	7.50	7.48	99.7	76.9	−3.9
Urine D	77.2	3.5	7.50	7.50	100.0	77.1	+ 1.6
Urine E	76.7	3.9	7.50	7.52	100.3	76.9	−1.5
Urine F	77.1	3.3	7.50	7.54	100.5	77.3	−2.6
Urine G	76.4	3.7	7.50	7.53	100.4	76.5	−1.3
Urine H	77.4	3.2	7.50	7.51	100.1	77.2	+ 2.6
Urine I	76.7	3.6	7.50	7.50	100	76.8.	−1.3
Urine J	77.1	3.1	7.50	7.52	100.3	77.3	−2.6
Average	76.9	3.6	7.50	7.44	98.8	77.0	

Phosphorescence Lifetime (τ)

For the sample containing 1.4 ag CPZ spot^{-1}, τ was determined by phosphorescence decay method (delay time 0.1–1.9 ms, gate time 2.0 ms). According to the method of Ref. [13], the regression equation of the delay curve could be expressed as ln I_p=5.4037−0.09834t, r was−0.9986, and τ was 10.2 ms. Long τ provided the possibility for the determination of CPZ by time-resolved RTP.

Interference Test

For the sample containing 3.6×10^{-15} g CPZ mL^{-1}, the allowed maximum concentrations of interfering species in human serum and urine were studied under the optimum measurement conditions. When the relative error (E_r) was within ±5%, the comparisons of the results between this method and those in Ref. [3] are listed in Table 4.

Scheme 1 A schematic illustrating the CPZ detection based on the reaction between CPZ and FITC

FITC   CPZ   FITC-CPZ

Drawn from Table 4, C and M of this method were higher than those in Ref. [3] showing good selectivity.

Sample Analysis

1.00 mL test solution was used to determine the content of CPZ in the samples with the experimental method and GC method and the results are listed in Table 5.

Seen from Table 5, the content of CPZ in human serum was 0.0305 $\mu g\ mL^{-1}$ which was less than death blood concentration (5 $\mu g\ mL^{-1}$) [4] by the oral dose of 100 mg (or 100000 μg) every day, and it could predict that the patients had no not life-threatening; and the residual CPZ in human urine was 76.9 $\mu g\ mL^{-1}$, indicating that 0.08% (76.9 μg/100000 μg) CPZ discharged from the urine.

Reaction Mechanism of CPZ and FITC

FITC could emit strong and stable RTP on PAM when Pb^{2+} was used as ion perturber. In the presence of CPZ, the RTP signal of FITC sharply enhanced with the λ_{em}^{max} red shifting for 19.3 nm, which indicated the formation of new compound. Ref. [14] reported the labelling compound FITC-chitosan was prepared between the reaction of the –NH$_2$ of chitosan and the –NCS of FITC. Thus, it was supposed that the new compound, that was FITC-CPZ (Scheme 1), was obtained in the reaction between the –NH$_2$ of CPZ and –NCS of FITC.

The structure of FITC-CPZ was analyzed with GC-MS. Results show that the appearance time of CPZ, FITC and FITC-CPZ were 16.2, 27.4 and 40.8 min, and mass charge ratio (m/z) of molecular ion peak were 318.1, 456.3 and 774.4 in the figure of GC-MS, respectively. Besides, the m/z of ion peaks was 86.2, 232.8 and 272.3, respectively. 272.3 (m/z) was the ion peak of $[M-N(CH_3)_2]$ that produced from CPZ molecular ion losing $N(CH_3)_2$. $[M-C_5H_{11}N]$ (m/z= 232.8) and $[C_5H_{12}N]$ (m/z=86.2) were the cyclo-N de-alkyl metabolites of CPZ. The facts above proved the possibility of CPZ reacting with FITC to form FITC-CPZ under the experimental conditions.

The group structure was changed due to the formation of –CS–NH– in the reaction between –NCS of FITC and –NH$_2$ of CPZ, which brought in the change of the π electronic density of carbon atoms (δ) in conjugate system [15]. According to semi-empirical method PM3, the π-electron density of carbon atom in the FITC and the FITC-CPZ conjugated ring was calculated. Results showed that the electron density between C$_9$ and C$_{14}$ was 0.090 for δ_{FITC} and 0.101 for $\delta_{FITC-CPZ}$, respectively. Compared with FITC, the π-electron density of carbon atom in the FITC-CPZ conjugated system increased, causing the RTP of FITC in the FITC-CPZ to sharply enhance (Fig. 1, curve 3.3′).

Besides, the ΔI_p of system has the linear correlation with the content of the CPZ. Thus, residual CPZ was determined by SSRTP.

Conclusion

A new method for the determination of trace CPZ has been established based on the academic thought that CPZ reacted with FITC to form FITC-CPZ compound, whose π-electron effect caused the RTP of FITC to sharply enhance. This sensitive method has been applied to the determination of residual CPZ in biological samples, and has provided a new way for the clinical detection.

References

1. Zhang ZB, Wang LJ, Meng FJ, Li HQ, Cheng G, Zhao Y, Kan JF, Zang YQ (2007) The protection of chlorpromazine on SD rats with brain injury. Misdiagnosis Misther 20:81–82
2. Yang QL, Liu ZF, Lu QM, Liu SP (2006) Resonance rayleigh scattering method for determ ina tion of some an ionic Surfactants with Chlorpromazine Hydrochloride as Probe. Chem J Chinese U 27:2281–2284
3. Cai FC, Chen ZH, Liu WB, Huang YM (2002) Determination of chlorpromazine hydrochloride by direct spectrofluorimetry. J Southwest Chin Nor Univ (Nat Sci) 27:739–741
4. Pharmacopoeia of the Peoples Republic of China II. (2005) Chemical Industry Press, Beijing, p 574
5. Pan HY, Han G (2006) Determination content of chlorpromazine hydrochloride injection with differential spectrophotometer. J North China Coal Med College 8:151–152
6. Ni YN, Qi ZB (2006) Differential kinetic spectrophotometric de-termination of chlorpromazine hydrochloride and promethazine hydrochloride by chemometric method. Spectrosc Spect Anal 26:1364–1367
7. Shi WB, Yang JD, Huang YM (2004) Ion-pair complex-based solvent extraction combined with chemiluminescence determina-tion of chlorpromazine hydrochloride with luminol in reverse micelles. J Pharmaceut Biomed Anal 36:197–203
8. Lu P, Qu ZN, Tan WQ, Zheng ZR, Jiang ZJ (2006) Study on determination of residues chlorpromazine hydrochloride in liver by gas chromatography-mass spectroscopy. Chin Animal Quarantine 23:30–31
9. Chen GZ (2007) Detection of chlorpromazine hydrochloride in urine by GC. Chinese J Health Lab Technol 17:2003–2004
10. Liu SL, Liu CH, Qin YJ, Zhao QQ, Liu XX, Li FL (2002) Simultaneous determination of sulpiride, clozapine and chlorpro-mazine in human plasma by HPLC. J Med Anal 22:365–368
11. He JD, Xia WS (2007) Study on labeing chitosan with fluorescein isothiocyanate. J Anhui Agi Sci 35:10939–10940
12. Liu JM, Zeng LQ, Li ZM, Gao F, Huang XM, Li FM, Lin HQ (2009) Solid substrate-room temperature phosphorimetry for the

determination of residual clenbuterol hydrochloride based on the catalysis of sodium periodate oxidizing eosine Y. Anal Chim Acta 638:69–74

13. Wei YS, Jin WJ, Yang Y, Liu CS (1998) Study on room temperature phosphorescence behavior of fluoranthene by laser induced time resolved technique. Chinese J Anal Chem 26:515–519

14. Liu XF, Guan YL, Yang DZ (2001) Antibacterial action of chitosan and carboxymethylated chitosan. J Appl Polym Sci 79:1324–1335

15. Chen GH, Huang CS (1988) A study of the chemiluminescence of some acidic triphenylmethane dyes. Tananta 35:625–631