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Sensitive chemiluminescence assay for risperidone in pharmaceutical preparations

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Abstract

A sensitive chemiluminescence (CL) method to determine the antipsychotic risperidone (RSP) is proposed, based on the catalytic effect of risperidone on the CL reaction between luminol and hydrogen peroxide in flow system. The increment of CL intensity was correlated with risperidone concentration in the range of 10 pg ml^{-1} to 1.0 ng ml^{-1} with a relative standard deviation of less than 5.0% ($n = 5$); and a limit of detection of 4 pg ml^{-1} (3σ). At a flow rate of 2.0 ml min^{-1}, the flow injection CL method exhibited both high sensitivity and excellent selectivity giving a throughput of 120 samples per hour. The proposed method was applied successfully to the determination of risperidone in pharmaceutical preparations.

Keywords: Risperidone; Chemiluminescence; Flow injection; Pharmaceuticals

1. Introduction

Risperidone (RSP) (Risperdal, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido [1,2-a] pyrimidin-4-one), a white to slightly beige powder, is an antipsychotic agent chemically classified as a benzisoxazole derivative, with serotonin-5-HT_2 and dopamine-D_2 antagonist activity [1]. Due to its favorable clinical effects and minimal extrapyramidal side effects [2], RSP is considered to be the first-line treatment option for schizophrenia and psychotic disorders which helps manage schizophrenia's "positive symptoms" such as visual and auditory hallucinations, delusions, and thought disturbances. RSP may also help in treating so-called "negative symptoms" such as social withdrawal, apathy, lack of motivation, and inability to experience pleasure [3,4].

An early method for the quantitative determination of RSP was a radioimmunoassay with specific antibodies against the active compounds [5]. A number of HPLC separation methods with electrochemical or ultraviolet detection after solid-phase extraction or multi-step liquid–liquid extraction [6–18] have been reported for the determination of RSP in biological fluids and pharmaceuticals since the early 1990s. Capillary zone electrophoresis for the determination of RSP was also described [19,20]. Since it was first reported by Albrecht in 1928 [21], the chemiluminescence (CL) resulting from the reaction of luminol and oxidants has been extensively studied and applied to the determination of a range of inorganic and organic species. For example, in a review Dodeigne et al. [22] reported CL methods as diagnostic tools and Palilis and Calokerinos [23] described analytical applications of CL reactions. Compared with other methods for the assay of RSP, CL method offers the advantages of simplicity of apparatus, low reagent consumption, higher sensitivities and higher sample throughput. However, to the best of our knowledge, no CL procedure has been used for the determination of RSP.

We have previously reported on the determination of isoniazid [24] and thiamine [25] using luminol–ferricyanide and luminol–periodate CL systems with flow injection (FI) analysis. In this paper we show that RSP greatly catalyzes the oxidation between luminol and hydrogen peroxide offering obvious increment on the original CL, providing a rapid and sensitive assay.

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2. Experimental

2.1. Reagents

All the reagents were of analytical grade; Water purified in a Milli-Q system (Millipore, Bedford, MA, USA) was used for the preparation of solutions. RSP was obtained from Shaanxi Institute for Drug Control. Hydrogen peroxide was purchased from Xi'an Chemical Reagent Plant. Luminol (Fluka, Biochemika) was obtained from Xi'an Medicine Purchasing and Supply Station, China.

Luminol was used as supplied to prepare a 2.5×10^{-5} M stock standard solution by dissolving 4.40 g luminol in 0.1 M sodium hydroxide in a 1.0 l calibrated flask. Hydrogen peroxide (33.3%) was prepared in water to give a final concentration of 0.1 M. A 5.0 M stock solution of sodium hydroxide was made by dissolving 100.00 g of the solid in distilled water and diluting to 500 ml in a calibrated flask. Finally, a stock standard solution of RSP was prepared from pure compound by dissolving 50.0 mg of substance in 50.0 ml of ethanol–water (50:50, v/v) to yield a concentration of 1.0 mg ml^{-1} and stored at 4 °C. Testing standards solutions were prepared daily by appropriate dilution of the stock solution.

2.2. Apparatus

The FI system used in this work is shown in Fig. 1. A peristaltic pump (Shanghai meter electromotor plant, model ND-15, 15 rpm) was used to generate the flows. PTFE tubing (1.0 mm i.d.) was used in the flow system. A six-way valve injected 100 μl solution of luminol. The CL emission cell is a twisty glass tubing (1.0 mm i.d., 15 cm length) in order to produce a large surface area exposed to the adjacent photomultiplier tube (PMT) (HAMAMATSU, model IP28). Extreme precautions were taken to ensure that the sample compartment and PMT were light tight. The CL signal produced in CL emission cell was detected without wavelength discrimination, and the negative high voltage (−680 V) was supplied to the PMT by a luminosity meter (Xi'an Keri Electron Device Ltd., model GD-1) connecting with a recorder (Shanghai Dahua Instrument and Meter Plant, model XWT-206).

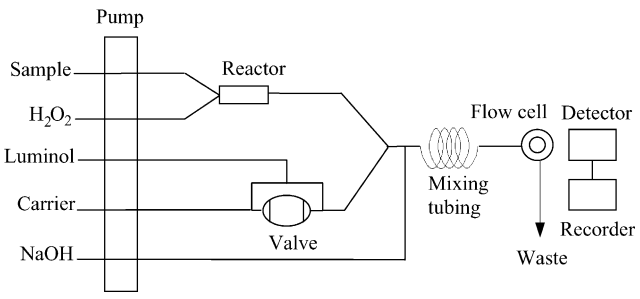

Fig. 1. Schematic diagram of the FI system for RSP determination.

2.3. Procedures

The carrier water and the reagents (luminol, hydrogen peroxide, sample and sodium hydroxide) were delivered at a flow rate of 2.0 ml min^{-1}. Once a stable baseline was recorded 100 ml of the solution of luminol was injected into the carrier stream, which was then mixed with the RSP and hydrogen peroxide streams. The mixed solution was delivered to the CL cell, and the peak height of the CL signal was detected with the PMT and the luminometer. The concentration of the sample was quantified by the increment of CL intensity ($\Delta I = I_s - I_o$), where I_o and I_s were CL signals in the absence and in the presence of RSP, respectively.

3. Results and discussion

3.1. CL intensity–time profile

Before the optimization of experimental conditions, the behavior of the CL reaction, carried out in the presence or absence of RSP, was investigated in a static system. The CL kinetic graph is illustrated in Fig. 2. As Fig. 2 shown, the RSP greatly catalyzed the CL reaction between luminol and hydrogen peroxide. Furthermore, in the presence of RSP a scintillescent CL signal was observed with an evident increment compared with that in the absence of RSP. The scintillescent CL intensity approached maximum at 1.2 s, which was 2 s faster than that in the absence of RSP, and then declined in the following 6 s.

3.2. Selection of oxidant

The characteristics of several oxidants, including permanganate, periodate, ferrocyanide, dichromate and hydrogen peroxide reacting with luminol in the presence of RSP were evaluated, and the results are shown in Table 1. It was found that RSP exhibited an enhancement for all the CL systems

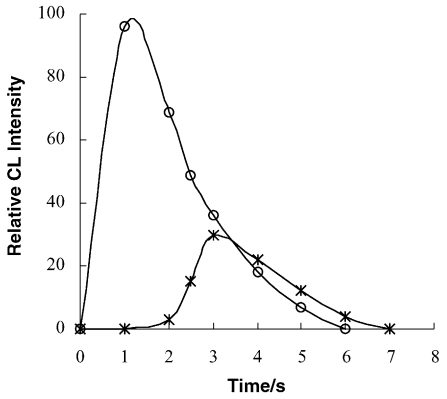

Fig. 2. CL intensity–time profile in static system: (∗) in the absence of RSP; (○) in the presence of RSP (0.1 ng ml^{-1}). The concentration of H_2O_2, luminol and NaOH were 1.0×10^{-5}, 1.0×10^{-7} and 0.025 M.

Table 1
Characteristics of different oxidants in static CL system[a]

Type of CL intensity	Relative CL intensity				
	$KMnO_4$	KIO_4	$K_3Fe(CN)_6$	$K_2Cr_2O_7$	H_2O_2
I_o	6	43	12	14	30
I_s	12	44	17	16	96
$I_s - I_o$	6	1	5	2	66

[a] The concentration for RSP, luminol, oxidants and sodium hydroxide were 0.1 ng ml^{-1}, 1.0×10^{-7}, 1.0×10^{-5} and 0.025 M, respectively.

tested, while the increments (ΔI) were different. The enhancement was dependent on the oxidant of the system, and the order of increase can be expressed as follows: $H_2O_2 \gg KMnO_4 > K_3Fe(CN)_6 > K_2Cr_2O_7 > KIO_4$. It was obvious that the luminol–hydrogen peroxide–RSP gave a maximum increase of CL intensity (ΔI), which suggested that the luminol–hydrogen peroxide system was more sensitive for the determination of RSP.

3.3. Effect of luminol, hydrogen peroxide and sodium hydroxide concentration

The effect of luminol concentration was investigated over the range from 5.0×10^{-9} to 5.0×10^{-7} M. The CL intensity increased steeply with an increase in luminol concentration up to 1.0×10^{-7} M, above which it decreased slightly. And ΔI reached maximum also at 1.0×10^{-7} M. Therefore, 1.0×10^{-7} M was selected for the analysis.

As regards the concentration of hydrogen peroxide, the intensity rose drastically when the concentration was increased up to 1.0×10^{-5} M, and then went down slowly from a higher concentration. The maximum ΔI was found at 1.0×10^{-5} M. Thus, 1.0×10^{-5} M was the optimum concentration used in subsequent experiment.

Owing to the nature of the luminol reaction, which is more favored under basic conditions, sodium hydroxide was introduced into the manifold through a flow line to improve the sensitivity of the system. A series of sodium hydroxide solutions of different concentration (0.01, 0.025, 0.05, 0.1, and 0.5 M, respectively) was studied. The concentration of sodium hydroxide versus CL intensity plot reached a maximum at about 0.025 M, and this concentration was employed in subsequent experiments.

3.4. Effect of flow rate and the length of mixing tubing

The CL intensity increased with increasing flow rate, and a 2.0 ml min^{-1} was chosen as a compromise between good precision and lower reagent consumption. The length of the mixing tube was also adjusted to yield maximum light emission in the cell. It was found that a 10.0 cm of mixing tube afforded the best results as regards sensitivity and reproducibility.

3.5. Performance of the system for RSP measurements

A series of standard solutions of RSP were injected into the manifold depicted in Fig. 1. The increase of CL intensity was found to be proportional with the concentration of RSP, offering linearity from 10 pg ml^{-1} to 1.0 ng ml^{-1}. The regression equation is $\Delta I_{CL} = 55.737 + 65.946 C_{RSP}$, and $r^2 = 0.993$ with the limit of detection 4 pg ml^{-1} (3σ). The relative standard deviations of five determinations were 4.02, 2.84 and 1.56% with RSP concentration of 0.01, 0.1 and 1.0 ng ml^{-1}, respectively. At a flow rate of 2.0 ml min^{-1}, the determination of the analyte could be performed in 0.5 min, including sampling and washing, giving a throughput of 120 per hour.

3.6. Interference studies

The interference of foreign substances was studied by adding increasing concentrations of these analytes to a 100 pg ml^{-1} RSP solution until a greater than 5.0% variation in CL intensity was achieved. The tolerable concentrations were over 30 μg ml^{-1} for glutin, barbiturate, oxalic acid, urea, and acetone, 7.0 μg ml^{-1} for methanol and ethanol, 1.2 μg ml^{-1} for albumin, 1.0 μg ml^{-1} for Cl^-, NO_3^-, Ac^-, I^-, SO_4^{2-}, PO_4^{3-}, $Cr_2O_7^{2-}$, borate, oxalate, tartrate and citrate, 0.7 μg ml^{-1} for globulin, 0.5 μg ml^{-1} for NH_4^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+} and myoglobin, 0.3 μg ml^{-1} for uric acid, and 0.5 ng ml^{-1} for Fe^{3+}, Fe^{2+}, respectively. The excipients commonly found in the pharmaceutical tablets, such as starch, lactose, cellulose and stearic acid, agar, talc, fructose and sucrose do not interfere with the determination at tolerable concentrations over 10 μg ml^{-1}.

4. Applications

4.1. Determination of RSP in pharmaceutical preparations

Three different preparations of RSP tablets were purchased from the local market. Not less than 10 tablets were weighed and then ground to fine powder. And then a sample equivalent to approximate 500 μg of RSP was weighed accurately, transferred into a 100 ml brown calibrated flask and made up to volume with ethanol–water (50:50, v/v). The sample was then diluted to a RSP concentration within the calibration range (0.01–1.0 ng ml^{-1}) without pre-treatment. Following the procedure described, the samples were determined by standard addition method into which a known quantity of RSP was added. The measured RSP contents (an average of five determinations) was listed in Table 2. The results obtained by the proposed method were 1.98, 2.01 and 2.02 mg per tablet with recovery varying from 95.1% to 108.5% and RSD of less than 3.0%, which were in good agreement with results obtained by differential scanning calorimeter (DSC).

Table 2
Results of RSP in pharmaceutical preparations[a]

Sample number	Added ($pg\,ml^{-1}$)	Found ($pg\,ml^{-1}$)	RSD (%)	Recovery (%)	Content of RSP in tablets (mg per tablet)	
					By proposed method	By DSC
1-1	0	9.9	2.71	95.2	1.98	1.95
	10	19.4	2.38			
1-2	0	29.9	2.17	95.1	1.99	2.01
	10	39.4	2.04			
1-3	0	9.8	2.31	96.1	1.96	1.98
	20	29.0	2.09			
2-1	0	20.2	2.56	104.8	2.02	1.97
	10	30.7	2.16			
2-2	0	29.9	2.09	108.5	1.99	1.98
	20	51.6	1.73			
2-3	0	20.2	2.57	102.4	2.02	2.01
	30	50.9	1.70			
3-1	0	9.9	2.68	97.7	1.98	1.97
	30	39.2	1.93			
3-2	0	30.1	2.03	101.0	2.03	2.03
	30	60.4	1.68			
3-3	0	20.4	2.24	95.6	2.04	2.01
	20	39.5	1.56			

[a] The average of five determinations.

5. Conclusions

Compared with other methods for the determination of RSP, the proposed flow system offers advantages in instrumental simplification, high sensitivity and reduced reagent consumption. Being conformed by its low detection limit and operational stability, the analytical procedure developed possessed good reproducibility, selection, precision and recovery in assay of RSP in pharmaceutical preparations. Preliminary experiments suggest that it may also be possible to apply the same techniques to the analysis of the drug in biological fluids such as plasma and urine.

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