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Determination of Baicalin in Traditional Chinese Preparation by High Performance Liquid Chromatography with Chemiluminescence Detection

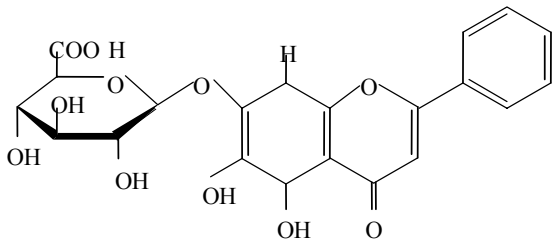
Er Bao LIU*, Hong Qing WEI, Xiu Li ZHAO, Xiao Xia LI, Feng Xian JIANG

School of Chemistry and Material Science, Shanxi Normal University, Linfen 041004

Abstract: A new method for the determination of baicalin with HPLC-CL was developed. The method was based on the chemiluminescence reaction between $KMnO_4$ and baicalin sensitized from HCHO. The linear range was 3.7×10^{-6}~9.8×10^{-5} mol/L with detection limit of 1.7×10^{-6} mol/L and the relative standard deviation was 2.5 % (Cs=6.6×10^{-5} mol/L, n=5). The method has been applied to the determination of baicalin in oral administration, injection, *Scutellariae radix* and granules with good results.

Keywords: RP-HPLC, chemiluminescence analysis, baicalin.

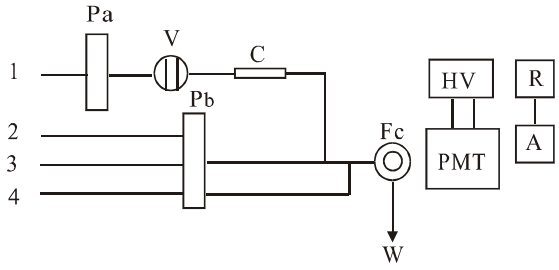
Herbal medicine, a form of complementary and alternative medicine, is becoming increasingly popular in the world[1]. *Scutellariae radix* is the root of *Scutellariabaica -lensis georgi*. The primary active constituent includes baicalin as follows:



Clinical studies showed that baicalin exhibited therapeutic functions of antifever, moistening aridity, anti-inflammatory and detoxifying [2] and it is also an anti-abortion agent as well as can scavenge free radicals and against oxidation[3]. So it is essential to develop a simple and reliable method for determination of baicalin in herb. Up to now, several methods have been developed, including high performance liquid chromatography[4], micellar electrokinetic capillary chromatography[5], and capillary electrophoresis electrochemistry[6]. At present, chromatography combining with CL

* E-mail: liueb@dns.sxtu.edu.cn

Figure 1 Schematic diagram of post-column HPLC with CL detection



1. mobile-phase; 2. $KMnO_4$; 3. HCHO; 4. HNO_3; Pa high pressure pump; Pb. peristaltic pump; C. column; V. injection valve; Fc. flow cell; W. waste; HV. high voltage; PMT. photo-multiplier tube; R. recorder; A. amplifier

detector has been developed greatly[7]. To our knowledge, the method of reverse high performance liquid chromatography combining with chemiluminescence detector for the determination of baicalin in herbal medicine has not been reported. In this study, a new method for the determination of baicalin with HPLC-CL was developed.

Experimental

The schematic diagram of post-column HPLC with CL detection is shown in **Figure 1**. Liquid chromatography experiments were carried out with a model HPLC-1050 system consisting of an automatic sampling valve with a work station (Hewlett Packard USA), a CL detection system with an eight-channel peristaltic pump (Xi'an Ruimai Electron Science and Technology Corporation, China). The standard solution of baicalin (1.23×10^{-4} mol/L) was prepared by accurately weighing and dissolving in water with a little methanol. The stock solutions were prepared fresh weekly and stored in refrigerator. All reagents were of analytical reagent grade and all solutions were prepared with water purified by Ultra-Pure Water System.

Solutions of $KMnO_4$ and HCHO were delivered with eight channel peristaltic pump at a rate of 0.8 mL/min, respectively. The solution of HNO_3 was added directly to the flow cell. The baseline was formed when HCHO and HNO_3 were added. The samples were separated using mobile phase at a rate of 0.8 mL/min, then entered into the CL system. The content of baicalin was calculated according to the equation $\Delta I=I_s-I_0$, where I_s was CL intensity with sample, I_0 was the CL intensity without sample.

Results and Discussion

Several reaction media such as HCl, H_3PO_4, HNO_3 and H_2SO_4 were studied in the concentration range of 0.1~4.0 mol/L. It was found that CL intensity was the highest in HNO_3. When the concentration of HNO_3 was increasing, CL signal was also increased. However, when the concentration of HNO_3 was more than 2.0 mol/L, CL intensity decreased with the increase of the concentration of HNO_3. A concentration of 2.0 mol/L

HNO_3 was found to be suitable for the detection of baicalin.

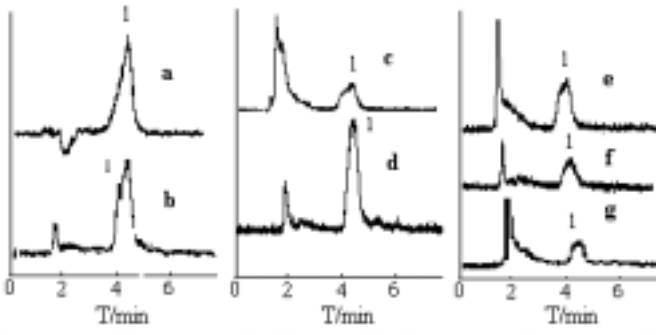
The effects of both potassium permanganate and formaldehyde on CL intensity were also studied. It was found that when the concentration of potassium permanganate was 9.0×10^{-5} mol/L, the CL signal ΔI was the highest, and the optimal concentration of formaldehyde was 5.0 % (V/V)

Under the conditions of potassium permanganate (9.0×10^{-5} mol/L), formaldehyde (5.0 %), nitric acid (2.0 mol/L) and baicalin (1.0×10^{-6} mol/L), it was found that CL intensity was the highest when the inside diameter of the pipes was 0.8 mm with a flow rate of 0.18 mL/min in each pipeline.

The mobile phase composition for HPLC was optimized. As with all post-column schemes, the selection of mobile phase in the post-column reaction was a critical step in the development of the method. Several kinds of mobile phase such as methanol-nitric acid and methanol-phosphoric acid have been tested for the separation of baicalin on RP-C_{18} columns. Among these mobile phases, methanol-phosphoric acid was found to be the best. Good separation was obtained using a mobile phase of methanol/0.3% phosphoric acid (47/53) with a flow rate of 0.8 mL/min at column temperature of 40℃.

Under the optimum conditions, a series of the standard solutions with a concentration range of 3.7×10^{-6}~9.8×10^{-5} mol/L were tested to determine the linearity. The determination limit was 1.7×10^{-6} mol/L on the basis of a signal-to-noise ratio of 3. The samples, injection of qingkailing, oral administrations of shuanghuanglian and kugan granules, pills of niuhuangjiedu and niuhuangshangqing, *Scutellariae radix*, were determined. The chromatograms of standard solution and samples were shown in **Figure 2**. Baseline separation for all analytes could be achieved within 5 min. The results were listed in **Table 1**, which indicates that this method is reliable, accurate and reproducible for all the analytes.

Figure 2 HPLC-CL chromatograms



(a) standard solution of 6.6×10^{-5} mol/L of *baicalin*, (b) injection of qingkailing, (c) oral administration of kugan granules, (d) *Scutellariae radix*, (e) oral administration of shuanghuanglian, (f) pill of niuhuangjiedu, (g) pill of niuhuangshangqing；1 baicalin, t_R=4.29 min

Table 1 Analytical results of samples (n=5)

Sample name	Obtained	Added/mg	Found/mg	Recovery/%	RSD/%
qingkailing injection	6.6 mg/mL	6.40×10^{-5}	6.38×10^{-5}	99.7	3.8
kugan granules	0.02 g/g	6.40×10^{-5}	7.03×10^{-5}	109.9	3.5
Scutellariae radix	0.26 g/g	6.40×10^{-5}	6.19×10^{-5}	97.7	3.2
shuanghuanglian	17.6 g/mL	6.40×10^{-5}	6.77×10^{-5}	105.8	4.3
niuhuangjiedu pill	48.8 mg/pill	6.40×10^{-5}	6.25×10^{-5}	97.7	5.6
niuhuangshangqing pill	22.8 mg/pill	6.40×10^{-5}	7.03×10^{-5}	109.9	2.8

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