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Stability of baicalin in biological fluids in vitro☆

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Abstract

The stability of baicalin in buffered aqueous solutions at different pHs and in biological fluids, including plasma, urine and tissue homogenates, were investigated in vitro. Structures of the degradation products of baicalin were elucidated by liquid chromatography–electrospray ion trap mass spectrometry and the degradation mechanism was proposed with the aid of electron paramagnetic resonance spectrometry. The results showed that the degradation of baicalin was pH- and temperature-dependent. The oxidation–reduction reaction intermediated by *phenoxyl radicals* is the major degradation process for baicalin in plasma and urine in vitro, whereas baicalin mainly undergoes hydrolysis and phase II metabolic pathways when spiked in tissue homogenates. It is found that acidification can stabilize baicalin in urine and plasma (the final pH adjusted to 3.0–4.0), and baicalin is relatively stable in tissues homogenized with methanol.

Keywords: Stability; Baicalin; Degradation products; Liquid chromatography/mass spectrometry; Electron paramagnetic resonance spectrometry

1. Introduction

As traditional Chinese medicines, many prescriptions, such as Huangqin-Tang are often used among Chinese patients for their special efficiency in inflammation, fever and allergic diseases cure [1]. Baicalin (Fig. 1) is one of the major active ingredients in Huangqin-Tang. The mechanism of the pharmacological action of baicalin was supposed to be based upon radical quenching and antioxidative effects [2].

During pharmacokinetic and metabolism studies, drug levels are commonly monitored in plasma, urine or tissue homogenates. In these studies, samples are generally collected, stored for a period of time, processed in some manner, and then analyzed. The degradation of labile compounds in biological fluids could yield misleading results from in vitro studies [3]. Due in part to the presence of 6,7-dihydroxyl groups in the benzene ring just like scutellarin [4], baicalin calls for the assessment of the stability during processing and storage of biological samples. Though, there were a lot of pharmacokinetic or metabolism studies on baicalin [5,6], only one report [7] was on the stability of baicalin (in buffered aqueous solutions at pH 2–8), and chalcone was proposed to be the degradation product at physiological pH.

To obtain validated methods to stabilize baicalin in biological fluids, studies on baicalin stability and decomposition were carried out in vitro in this paper.

2. Experimental

2.1. Chemicals and solvents

Baicalin (purity, >98.3%) was purchased from Shifang Co. (Sichuan, China). Baicalein (Fig. 1; purity, >99.5%) and 6-OCH_3–baicalein (purity, >99.5%) were obtained in our lab. Daidzein (Fig. 1; purity, >99.3%) was purchased from Huike Co. (Shanxi, China). Expected structures of all the standards were confirmed by MS and NMR spectroscopy. β-Glucuronidase (with arylsulfatase activity, partial purified,

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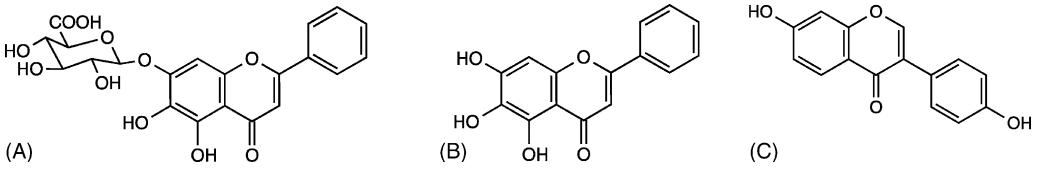


Fig. 1. Molecular structures of baicalin (A), baicalein (B) and daidzein (internal standard, C).

G-4259) was purchased from Sigma Chemical Co. (St. Louis, MO). Other reagents used were HPLC or reagent grade. Buffered aqueous solutions at pH 7.4 and 9.0 were prepared according to Chinese Pharmacopoeia 2000. The aqueous buffer at pH 7.4 consisted of 0.05 M KH_2PO_4 and 0.04 M NaOH. The aqueous buffer at pH 9.0 was obtained as follows: 3.09 g of boric acid was dissolved in 500 mL of KCl (0.1 M) solution, then 210 mL of NaOH (0.1 M) was added.

2.2. Liquid chromatography–electrospray ion trap mass spectrometry (LC/MS^n) and electron paramagnetic resonance spectrometry (EPR) analysis

The HPLC system (Shimadzu, Kyoto, Japan) was equipped with a Finnigan LCQ ion trap mass spectrometer (San Jose, CA) via an electrospray ionization interface. A Diamonsil C_{18} column (particle size 5 μm, 20 cm × 4.6 mm i.d., Dikma, China) was used for sample separation. Mobile phase consisting of methanol–water–formic acid (60:40:1, v/v/v) was used at a flow rate of 0.5 mL/min for the analysis. The ionization was realized by applying spray voltage of 4.25 kV and capillary temperature of 180 °C. Nitrogen was used as both the sheath gas at a flow rate of 0.75 L/min and auxiliary gas at a flow rate of 0.15 L/min. MS^n spectra were obtained in the positive ion mode, using helium as collision gas. The mass spectrometer was operated in selected reaction monitoring mode (SRM) recording the transitions from the respective $[M+H]^+$ ions to the following product ions: baicalin m/z 447 → m/z 271 and daidzein m/z 255 → m/z 199. Data were analyzed by Xcalibur software (Version 1.2, Finnigan).

Bruker ER 200D electron paramagnetic resonance spectrometer was used for analyzing structures of the degradation products when baicalin introduced into the aqueous solution (pH 9.0). Microwave frequency was set at 9.67 GHz and microwave power was set at 20 mw. Modulation frequency, modulation intensity and field-sweep width was applied at 100 kHz, 0.125 and 20 mTorr, respectively.

2.3. Animals and sample collection

Male Wistar rats weighing 230 ± 50 g were supplied by Lab Animal Center of Shenyang Pharmaceutical University (Grade II, Certificate No. 042). Fresh urine, plasma and tissue samples (including liver, stomach, intestine and kidney) were collected below 4 °C prior to experiments. Tissue homogenates were obtained by homogenizing the tissues (1 g) with 2 mL of ice-cold physiological saline or methanol.

2.4. Stability of baicalin in buffered aqueous solutions at different pHs, in biological fluids and under anaerobic conditions

The aqueous buffer at pH 7.4 was selected to simulate plasma and the aqueous buffer at pH 9.0 was used to simulate urine. Stability curves of baicalin were established based on the logarithm value of the remaining drug (the initial concentration of baicalin was presumed to be 100 unit) versus time. Each set of experiments was conducted in triplicate. Incubations were terminated by adding 500 μL of methanol–0.3 M hydrochloric acid (10:1, v/v; containing IS, 10.0 μg/mL). The stability of baicalin in the termination medium (e.g. buffer at pH 7.4-methanol–hydrochloric acid system, pH 3.0–4.0) was evaluated.

2.4.1. Stability of baicalin in buffered aqueous solutions at pH 7.4 and 9.0, in organic solvents and anaerobic conditions

Baicalin at the concentration of 100 μg/mL was incubated in two buffer systems at pH 7.4 and 9.0 for 2 h. This experiment was performed at 25, 35 and 45 °C, respectively. The stability of baicalin in buffers (at pH 7.4 and 9.0; 25 °C) containing 0.2% $Na_2S_2O_4$ as antioxidant was also evaluated. Aliquots of 100 μL taken at predetermined time of different intervals (25 °C: 0, 0.33, 0.67, 1.0, 1.5 and 2.0 h; 35 °C: 0, 0.25, 0.5, 0.75 and 1.0 h; 45 °C: 0, 0.17, 0.33, 0.5, 0.75 and 1.0 h). After incubations, aliquot of 10 μL solution were immediately injected into the liquid chromatograph for LC/MS^n analysis.

The stability of baicalin in methanol and ethyl acetate was investigated for a week at ambient temperature (25 °C). The initial concentration of baicalin in methanol or ethyl acetate was 100 μg/mL. Aliquots of 100 μL solution were taken after a week and incubations were terminated. An aliquot of 10 μL solution was injected for LC/MS^n analysis.

The stability of baicalin (in the buffer at pH 7.4) under anaerobic conditions (solution saturated with argon) was investigated for 12 h. The initial concentration of baicalin was 100 μg/mL. Aliquots of 100 μL solution were taken after 12 h, and mixed with 500 μL of termination fluid (methanol–0.3 M hydrochloric acid system containing IS). An aliquot of 10 μL solution was injected for LC/MS^n analysis.

2.4.2. Stability of baicalin in rat plasma and urine

Fresh rat blood was collected with ice-cold test tubes containing heparin and plasma was immediately obtained after

centrifugation (3000 × *g*, 5 min) at 4 °C. Baicalin at the concentration of 100 μg/mL was mixed with plasma and kept at 25 °C for 1 h. Aliquots of 100 μL plasma sample were taken at predetermined time of 0, 0.17, 0.33, 0.5, 0.67, 0.83 and 1.0 h, and mixed with 500 μL of termination fluid. The mixture was vortexed for 1 min, and centrifuged at 3000 × *g* for 5 min.

Fresh rat urine was collected and filtered through filtration membrane (0.45 μm). Baicalin at the concentration of 100 μg/mL was mixed with urine and kept at 25 °C for 1 h. Aliquots of 100 μL urine sample were taken at predetermined time of 0, 0.25, 0.50, 0.75 and 1.0 h. The solutions were mixed with 500 μL of termination fluid.

An aliquot of 10 μL supernatant (plasma) or fluid (urine) was injected into the liquid chromatograph for LC/MS^n analysis.

2.4.3. Stability of baicalin in rat tissue homogenates

Rat tissue homogenates (in physiological saline), including stomach, intestine, kidney and liver were incubated with baicalin at the initial concentration of 100 μg/mL at 25 °C. Aliquots of 100 μL liver homogenate incubation sample were taken at predetermined time of 0, 0.08, 0.17, 0.25, 0.42, 0.67 and 1.0 h, and mixed with 500 μL of methanol. Other tissue samples were taken only at time of 0.25 h to investigate the degradation of baicalin. The mixture was vortexed for 1 min, and centrifuged at 3000 × *g* for 5 min. In this experiment, the stability of baicalin in liver homogenate was evaluated in detail for the high-enzymic activity in liver, which continues in the sample after sample collection. An aliquot of 10 μL supernatant was injected into the liquid chromatograph for LC/MS^n analysis. The stability of baicalin in liver homogenized with methanol was investigated for 24 h.

2.5. Calibration curves

Baicalin and daidzein (internal standard, IS) standards were dissolved in methanol. For calibrator preparation, 100 μL buffered aqueous solution at pH 7.4 or biological fluids spiked with various concentrations of baicalin was added with 500 μL of methanol–0.3 M hydrochloric acid (10:1, v/v; containing IS, 10.0 μg/mL) to afford standards with the final concentration of 0.1, 0.2, 0.5, 2.5, 10.0 and 50.0 μg/mL, respectively. Calibration curves were established based on the ratio of peak area (baicalin/IS) versus concentration by using weighted ($w = 1/c$) linear regression analysis. A good linear relationship was obtained for baicalin in the concentration range of 0.1–50.0 μg/mL in both buffered aqueous solutions and biological fluids (including plasma, urine and liver homogenates). The lower limit of quantification (LLOQ) of baicalin was 0.1 μg/mL. Typical equations of the calibration curves of baicalin were as follows: buffered aqueous solution at pH 7.4: $y = 1.230 \times 10^{-3} + 5.431 \times 10^{-2}x$, $r = 0.9992$; plasma: $y = 1.170 \times 10^{-2} + 2.192 \times 10^{-2}x$, $r = 0.9912$; urine: $y = 1.675 \times 10^{-2} + 4.793 \times 10^{-2}x$, $r = 0.9972$; liver homogenates: $y = 2.764 \times 10^{-3} + 9.832 \times 10^{-2}x$, $r = 0.9968$. Where y represents the ratio of baicalin peak area to that of daidzein and x the concentrations of baicalin. Representative chromatograms of blank plasma samples, blank plasma sample spiked with baicalin (0.1 μg mL^{-1}) and IS (10.0 μg mL^{-1}), and rat plasma samples at 2 h after baicalin spiked in plasma at 25 °C are presented in Fig. 2.

2.6. Method validation

The method was evaluated through intraday and interday analysis of precision and accuracy.

The accuracy and precision of the method were assessed by determining quality control (QC) samples using six replicate preparations of plasma (or buffered aqueous solution at pH 7.4) samples at three concentration levels (0.1, 0.5 and 10.0 μg/mL) on three separate days. The precision and accuracy of this method indicated that all coefficients of variation (CVs) were below 10.0%. Table 1 summarizes the intra- and interday precision and accuracy for baicalin evaluated by assaying the quality control (QC) samples of rat plasma as a representative example. The above results demonstrated that the values were within the acceptable range and the method was accurate and precise.

2.7. Structures of degradation products of baicalin

When direct MS^n analysis (only volatile solvents are suitable for this type of mass spectrometer) was performed,

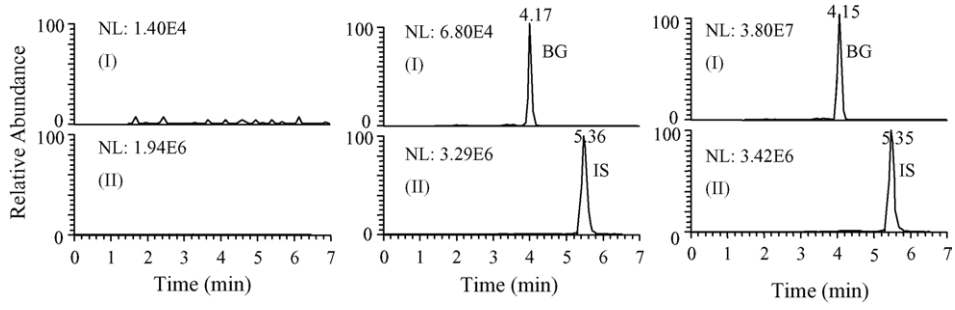


Fig. 2. Representative SRM chromatograms of baicalin (BG): left, a blank rat plasma sample; middle, a blank rat plasma sample spiked with BG (0.1 μg mL^{-1}) and IS (daidzein, 10.0 μg mL^{-1}); right, a rat plasma sample at 2 h after BG spiked in plasma at 25 °C. (I) monitoring SRM of BG: m/z 447 → m/z 271; (II) Monitoring SRM of IS (daidzein): m/z 255 → m/z 199.

Table 1
Summary of precision and accuracy from quality control (QC) samples of rat plasma extracts of baicalin (n = 3 days, six replicates per day)

Added concentration ($\mu g\,mL^{-1}$)	Found concentration ($\mu g\,mL^{-1}$)	Accuracy (%)	Precision (%)	
			Intra-run	Inter-run
0.10	0.097	97.0	3.6	4.9
0.50	0.51	102.0	4.2	9.0
10.0	10.01	100.1	6.5	6.1

baicalin was dissolved in ammonia water (0.01%, v/v; pH 9.0) instead of biological fluids or the buffer at pH 9.0. Samples at time of 0.17, 2.0 and 12.0 h after incubation were taken for MS^n analysis. The same sample was analyzed by electron paramagnetic resonance spectrometry (EPR).

Tissue samples of baicalin were analyzed by LC/MS^n. Control samples of baicalin incubated with liver homogenates pretreated with boiling water were set meanwhile. For enzymic hydrolysis, 50 μL of tissue homogenates was mixed with 50 μL of β-glucuronidase (1000 U/mL in pH 5 acetate buffer), and incubated at 37 °C for 8 h under anaerobic conditions and protected from light. After hydrolysis, 200 μL of methanol was added. The aqueous-methanol supernatant was for LC/MS^n analysis.

3. Results and discussion

The preparation of baicalin calibration curves was only used to show that the response of baicalin detected by LC/MS^n was linear in the range of 0.1–50.0 μg/mL. The possible shift of concentration of baicalin caused by nonlinear response was avoidable. Relevant validation data, such as specificity and precision were also described in detail in this paper. Baicalin was found stable in methanol and ethyl acetate for at least a week at ambient temperature. Anaerobic conditions could also stabilize baicalin at pH 7.0–9.0. Baicalin and daidzein were relatively stable for at least 2 h in the termination medium (pH adjusted to 3.0–4.0 by methanol–hydrochloric acid) at ambient temperature. It was proposed due to that acidic environment can prevent reactions intermediated by radicals [8]. Samples were determined within 2 h once incubation was terminated in this experiment. The degradation rate constants (k) and half-lives ($t_{1/2}$) of baicalin in buffered aqueous solutions at different pHs and in biological fluids are given in Table 2 according to the first-order kinetics equation.

3.1. Stability of baicalin in buffered aqueous solutions at different pHs and biological fluids

The stability profiles of baicalin in buffered aqueous solutions (at different temperatures) at pH 7.4 and 9.0 are shown in Fig. 3. In buffered aqueous solutions at pH 7.4 and 9.0, the Arrhenius plots of the degradation reaction exhibited negligible deviation from a straight line over the whole experimental temperature range. The results showed that the degradation of baicalin was pH- and temperature-dependent. The half-life of baicalin was 1.75 h at pH 7.4, while 0.83 h at pH 9.0. Though constants k and $t_{1/2}$ were perhaps related to concentration [9], relatively rapid degradation of baicalin in these two solutions was observed. Baicalin was stable in buffered aqueous solutions containing antioxidant for 1 h (pH 7.4) and 0.5 h (pH 9.0), which suggests that the reaction mechanism belongs to oxidation–reduction reaction. But baicalin began to degrade after a short time (after 1 h at pH 7.4), which was supposed to be caused by the exhaustion of the antioxidant.

The stability profiles of baicalin in plasma, urine and liver homogenate at ambient temperature (25 °C) are shown in Fig. 4. The degradation profile of baicalin in urine was similar to that in the aqueous buffer at pH 9.0, and the profile in plasma was similar to that in the aqueous buffer at pH 7.4. The result indicated that pH, but not the matrix of plasma or urine played an important role in the degradation of baicalin.

Table 2
Degradation parameters of baicalin in buffered aqueous solutions and biological fluids

Medium	First-order kinetic equation	Correlation coefficient (r)	Degradation constant (k)	Degradation half-life ($t_{1/2}$, h)
*pH 7.4 (25 °C)	$\log C = -0.1724t + 1.9974$	0.9995	0.397	1.75
*pH 7.4 (35 °C)	$\log C = -0.5730t + 1.9912$	0.9990	1.343	0.52
*pH 7.4 (45 °C)	$\log C = -1.8331t + 1.9767$	0.9982	4.223	0.16
*pH 9.0 (25 °C)	$\log C = -0.3609t + 1.9928$	0.9994	0.831	0.83
*pH 9.0 (35 °C)	$\log C = -1.0629t + 2.0141$	0.9977	2.448	0.30
*pH 9.0 (45 °C)	$\log C = -2.0602t + 2.0086$	0.9988	4.626	0.15
Plasma (25 °C)	$\log C = -0.1311t + 2.0003$	0.9975	0.302	2.30
Urine (25 °C)	$\log C = -0.3726t + 1.9827$	0.9951	0.858	0.81
Liver homogenate (25 °C)	$\log C = -2.2270t + 1.9998$	0.9994	5.129	0.14

*Buffered aqueous solution.

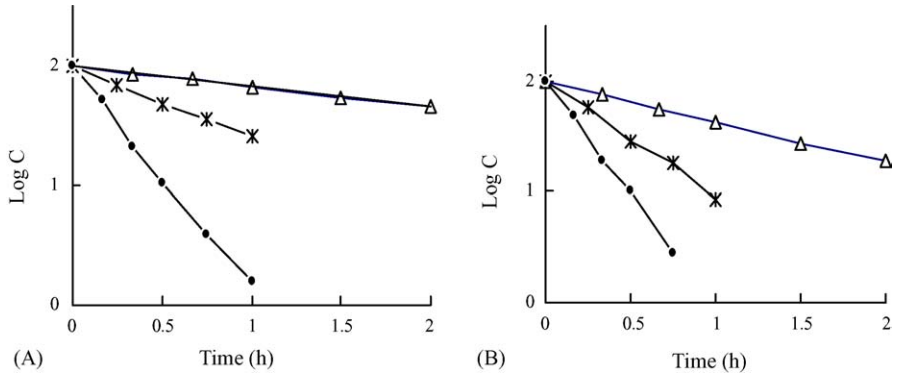


Fig. 3. The stability of baicalin in different buffers at different temperatures: (A) pH 7.4; (B) pH 9.0; (△) 25 °C; (✱) 35 °C; (●) 45 °C.

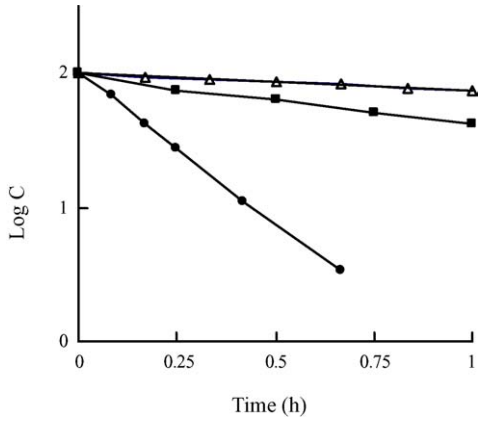


Fig. 4. The stability of baicalin in rat plasma, urine and liver homogenate at 25 °C: (△) plasma; (■) urine; (●) liver homogenate.

The fresh urine collected from the metabolic cage was found at about pH 9.0, which was higher than its normal value, pH 7.5–8.3. It was possibly due to the evaporation of water in urine during the process of collection. Baicalin was found half of its concentration after only 7 min spiked in the liver homogenate. The degradation may be due to the enzymes in liver. The degradation rates of baicalin spiked in other tissues were all higher than that in plasma, which suggested that other factors (besides pH) counted in the reaction, such as gastric fluid or enzymes in the tissues.

3.2. Structures of degradation products of baicalin in biological fluids

The profile of baicalin in ammonia water at pH 9.0 at 10 min analyzed by EPR (Fig. 5) showed that the degradation product was a radical with an apparent EPR signal. The single broad line EPR (g = 2.0045) signal corresponded to phenoxyl radical intermediates as previously reported for other compounds [10]. Because the g value was higher than 2.0023 (the value of carbon-centered radical), we assumed a radical structure where the unpaired electron resides mostly on the oxygen and to a less extent on the carbon atoms. Phenoxyl radicals resulted from a one-electron oxidation pathway of compounds. A high number of hydroxyl substituents facilitated unpaired-electron delocalization, leading to a dipolar configuration where multiple mesomeric structures could exist [10]. This was probably the reason why baicalin gave a strong unresolved EPR spectrum corresponding to the envelope of several different spectra. On the other hand, because of the low sensitivity of the EPR method, a high concentration of 200 μM of baicalin was necessary to detect phenoxyl radical intermediates. At this high-concentration, the hyperfine splitting was not observed. Based on the result of MS^n, ion at m/z 446 instead of the precursor ion of baicalin (m/z 447) was detected at the same moment, the initial degradation product of baicalin was not chalcone or quinone as Ref. [7] showed. The degradation from baicalin to its radical form was found reversible after adding proper acid (pH adjusted

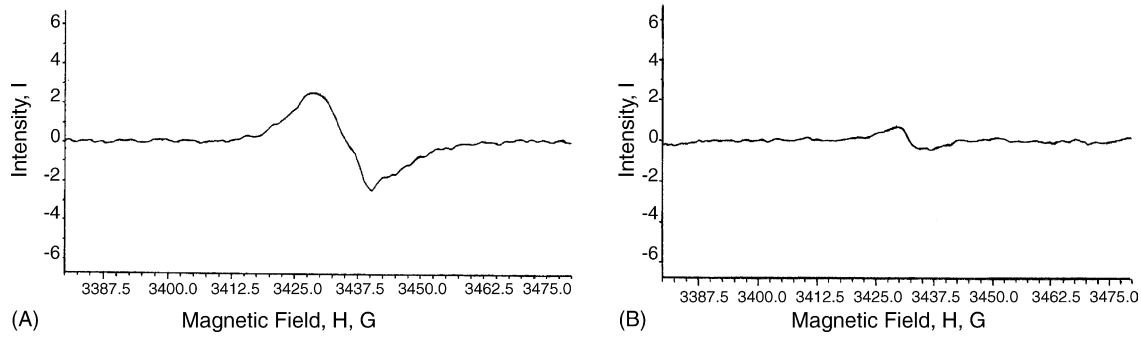
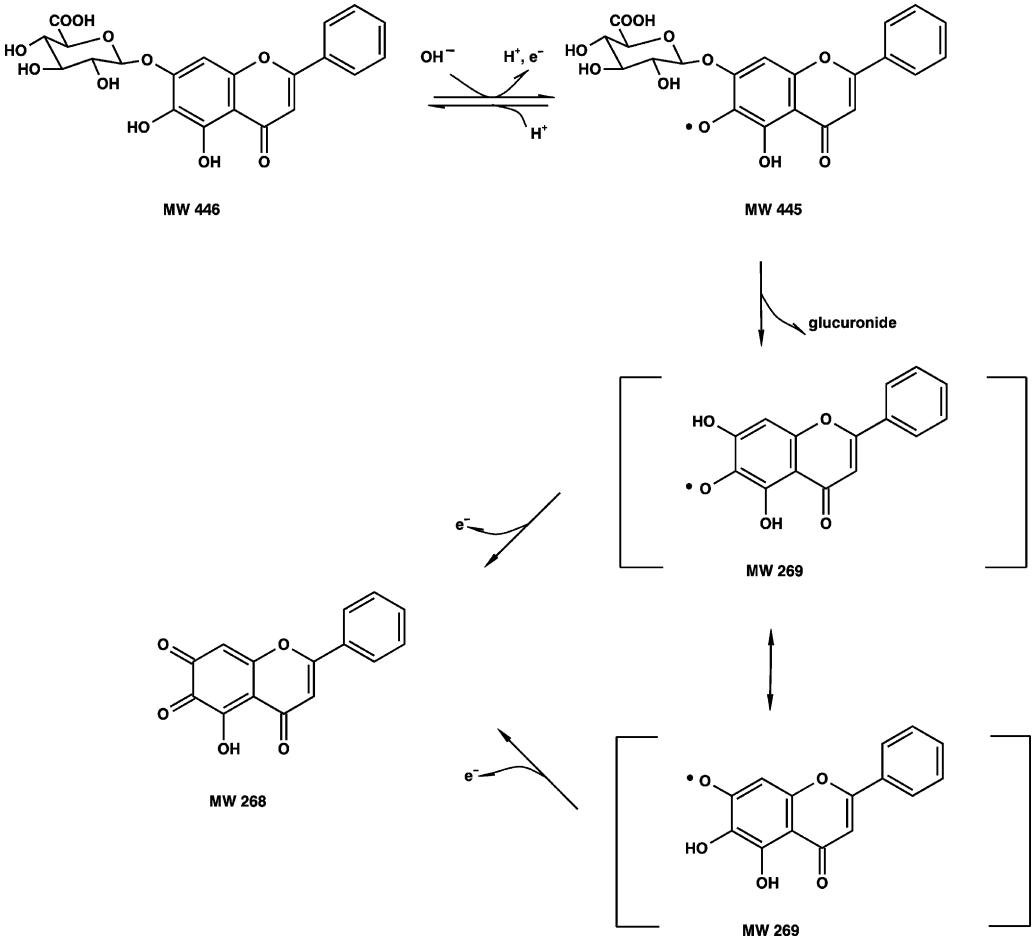


Fig. 5. The electron paramagnetic resonance spectra of baicalin at different time after addition of ammonia water (0.01%, v/v; pH 9.0): (A) 10 min; (B) 2 h.



Scheme 1. The proposed degradation profile of baicalin in the aqueous solution at pH 9.0.

<3) or $Na_2S_2O_4$ as antioxidant (0.2%). The intensity of the EPR signal declined at 2 h and ion at *m*/*z* 269 was detected by MS, which suggested that further transformation of baicalin radicals happened. Based on the results of EPR and MS^n, the final degradation product of baicalin at 2 h was supposed to be quinone. The mechanism in decreasing phenoxyl radical levels could be due to the transformation of phenoxyl radicals into quinonoid compounds, corresponding to the final two-electron oxidation products [10]. The degradation scheme of baicalin in biological fluids is shown in Scheme 1.

The stability of the intermediate radicals depends on their delocalization of the unpaired electron. Therefore, the phenoxyl radicals exhibit higher stability than flavonoxyl radicals [11]. Some authors reported that phenoxyl radicals were transient, except at higher pH [10]. Partly because of the short lives of radical species [12] and less radicals formed, no obvious EPR signal was observed in the aqueous buffer at pH 7.4. Due to the similarity of their structures, scutellarin and baicalein (the aglycone of baicalin) could also undergo this degradation reaction at pH 9.0 (data not shown).

The degradation products in tissue homogenates were analyzed by LC/MS^n and confirmed by the comparison of LC behavior and MS^n data with reference standards. In the incubation samples of tissue homogenates spiked with baicalin, several main degradation products were detected, including baicalein (*m*/*z* 271), 6-OCH_3-baicalein (*m*/*z* 285) and three phase II metabolites. They were supposed to be 6-*O*-β-glucopyranuronoside (*m*/*z* 447), baicalein 6,7-di-*O*-β-glucopyranuronoside (*m*/*z* 623) and 6-*O*-β-methyl-baicalein-7-*O*-β-glucopyranuronoside (*m*/*z* 461). The degradation products of baicalin in fresh liver homogenates as a representative example detected by LC/MS^n are shown in Fig. 6. The result indicated that liver enzymes played an important role in the degradation of baicalin in liver homogenates. These phase II compounds could be hydrolyzed to baicalein or 6-OCH_3-baicalein by β-glucuronidase. These phase II metabolites have been detected in rat bile [6].

3.3. Methods for the sampling and storage of biological samples of baicalin

Baicalin was found relatively stable in organic solvents or when kept acidic in solutions, especially at pH 3.0–4.0 (acidified by hydrochloric acid in this experiment). Though baicalin was also stable under the condition free from oxygen, the anaerobic condition was often difficult to realize. Based

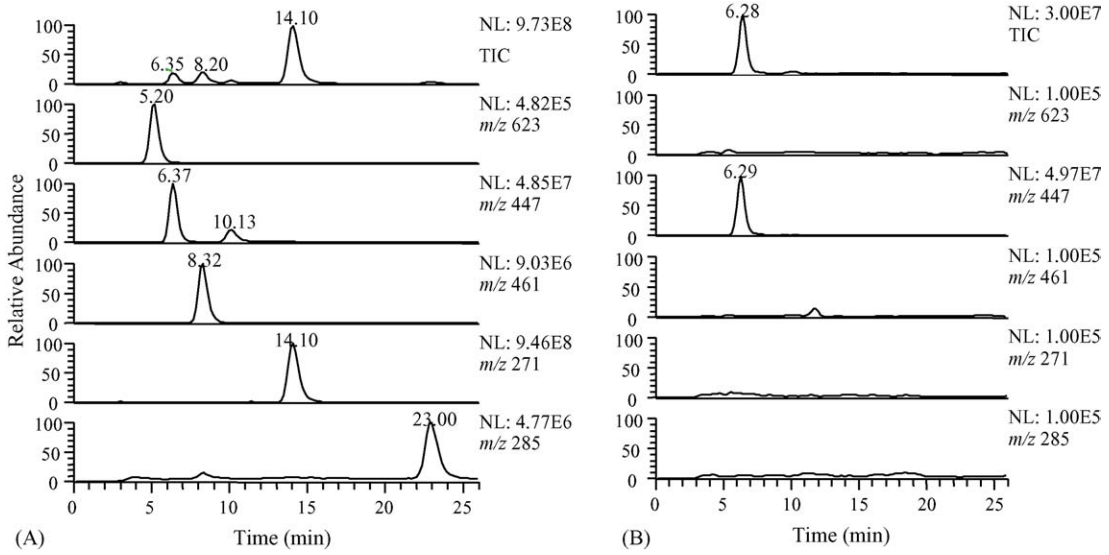


Fig. 6. The profile of baicalin incubated with liver homogenate by LC/MS^n: (A) fresh liver homogenate in physiological saline; (B) liver tissue pretreated by boiling water.

on the stability experiments and the degradation mechanism, we evaluated the simple methods for the pretreatment and storage of biological samples.

Blood was collected with ice-cold test tubes or on ice. Plasma was obtained after centrifuging at low temperature (4 °C) during a short time (3000 × *g*, 5 min). Then plasma was immediately acidified with hydrochloric acid (adding 10 μL HCl to 1 mL plasma, pH 4.0) and stored at −20 °C till use. Urine samples were collected with plastic tubes, in which hydrochloric acid was added (adding 100 μL HCl to 1 mL urine, pH 4.0). Then urine samples were stored at −20 °C. Tissues taken off the animals were placed in ice-cold water at once. Proper aliquots of tissue slices were immediately homogenized with methanol (1:2, g/mL) after weighing and cutting. The supernatant was stored at −20 °C prior to analysis.

The biological samples after being pretreated according to above remained stable at ambient temperature for at least 24 h and stable for 2 months at −20 °C. These methods for pretreatment and storage of biological samples of baicalin could provide us a leisure time during sample analysis.

Though, there were a lot of papers [13–16] on the metabolism or pharmacokinetics of baicalin, the stability of baicalin in the sampling process was not considered in these reports. Only the stability of baicalin in plasma samples, which has been prepared for analysis, was mentioned [13]. It was found that baicalin was stable in plasma extracts reconstituted by acidic mobile phase. Additionally, baicalin was stable in plasma precipitated by acetonitrile instead of methanol [14], which was probably due to that methanol containing buffered aqueous solution was the medium to transfer electron, then to induce the degradation of baicalin. We observed that baicalin was stable in tissues homogenized by methanol, because of the presence of minor buffered aqueous solution in homogenates. In the pharmacokinetic study of baicalin, the total amount of baicalin after enzymatic hydrolysis was usually determined. The enzymatic hydrolysis procedure was often conducted under anaerobic conditions and protected from light [5,15]. The mechanism to stabilize baicalin in the hydrolysis interval (about 8 h) was that the production of phenoxyl radicals catalyzed by oxygen and light was inhibited. The methods to stabilize baicalin in tested samples by acidification or keeping anaerobic are in correspondence with the result of the present study.

4. Conclusions

The forgoing discussions indicate that pH and temperature may result in the degradation of baicalin in biological fluids after samples have been collected and during sample processing. The degradation of baicalin in urine and plasma in vitro is the result of the oxidation–reduction reaction intermediated by phenoxyl radicals. Baicalin can undergo hydrolysis and phase II metabolic pathways when spiked in tissue homogenates in vitro. Thus, maintaining a stable acidic pH in plasma and urine samples is essential when attempting to collect, process and store biological samples of baicalin. Organic solvents, such as methanol can be used to homogenize tissues of baicalin. The results may also be of some practical utility for other baicalin analogues, such as baicalein and scutellarin.

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