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Simultaneous Determination of Baicalin and Berberine Hydrochloride in Fufangkangkui Suspension by HPLC

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A high-performance liquid chromatography method with diode array detection was developed for simultaneous analysis of baicalin and berberine hydrochloride in Fufangkangkui suspension, a traditional Chinese medicine being prescribed widely for antibacterial, antiviral and immunostimulant activity. A relatively simple extraction procedure was employed and optimized and separation of the components was obtained within 0.5 h using an Agilent ZORBAX Eclipse XDB-C_{18} column under gradient elution with acetonitrile and a buffer containing 6 mmol/L KH_2PO_4 solutions (pH = 4.6). All calibration curves showed good linear correlation ($r^2 > 0.9997$) within the test ranges. Validation proved that the repeatability of the method was good and recovery was satisfactory. The HPLC method was able rapidly and efficiently to analyze baicalin and berberine hydrochloride in Fufangkangkui suspension. In conclusion, these results demonstrated that the method proposed was useful for the analysis and quality consistency evaluation of Fufangkangkui suspension.

Keywords: Baicalin, Berberine hydrochloride, Fufangkangkui suspension, HPLC.

INTRODUCTION

Chinese herbal medicine has attracted considerable attention all over the world. Hundreds of traditional Chinese medicines (TCMs) are utilized to treat various diseases, which greatly promote the development of corresponding, effective and precise analytical methods[1]. It is no doubt a remarkable progress that many studies have focused on simultaneous determination of multiple constituents in traditional Chinese medicine[2,3].

Fufangkangkui suspension was produced by the first people's hospital of Bengbu city, Anhui province, China. It consists of rhizoma coptidis, scutellaria baicalensis and cortex phellodendri as the most classical traditional Chinese medicine prescriptions. It is widely used for treating inflammation, expelling of toxin, to ease pain and promote healing of ulcer surface. Baicalin and berberine hydrochloride are two representative bioactive constituents in the prescription. So far, Fufangkangkui suspension has not been a complete quality control standards and has not been a method to detect the inside of the active ingredient. It is widely accepted that therapeutic effect of traditional Chinese medicine (TCM) is usually based on multiple essential components or their combination rather than any single component. Considering the above problems, it is absolutely necessary to develop a satisfactory method for simultaneous detection of as many active compounds in Fufangkangkui suspension as possible to ensure its effectiveness and safety[4].

According to a report in the literature, the development of an HPLC method for simultaneous determination of baicalin and berberine hydrochloride have been applied to the detection of many traditional Chinese medicines, however, there was no methods reported for simultaneously determining baicalin and berberine hydrochloride in Fufangkangkui suspension[5,6]. Hence the aim of the study was focused on finding a method for the simultaneous determination of baicalin and berberine hydrochloride in Fufangkangkui suspension. In our study, baicalin and berberine hydrochloride was successfully separated on XDB-C_{18} column with a mixture of 6 mmol/L KH_2PO_4 solutions-acetonitrile. The retention times of baicalin and berberine hydrochloride were only 9.724 and 18.467 min, the peak asymmetries were 1.08 and 1.06, so it was better than others described before. Furthermore, the results indicated that the linearity, intra-day precision, inter-day precision and reproducibility were good. In sum, this method was rapid and simple, which could be used for simultaneously determining baicalin and berberine hydrochloride in Fufangkangkui suspension.

EXPERIMENTAL

HPLC was performed on an Agilent 1260 series HPLC system, HPLC grade methanol was purchased from Merck Company (Merck, Darmstadt Germany), deionized water used in all experiments was prepared by a Milli-Q Biocel Ultrapure

Water system (Millipore, MA, USA). Standards of baicalin and berberine hydrochloride were obtained from the National Institute for Food and Drug Control (Beijing, China). The sample of Fufangkangkui suspension was obtained from the first people's hospital of bengbu, Anhui province, China. All other chemicals were of analytical reagent grade.

Preparation of standard and sample solution: The stock solutions of baicalin and berberine hydrochloride were prepared by precisely weighing the reference standards and dissolving in 70 % ethanol to yield the concentrations of 54.5 and 101.01 μg/mL. The stock solutions was further diluted to make working solution. All the solutions were stored in the refrigerator at 4 °C before HPLC analysis. We weighted about 5 g Fufangkangkui suspension samples in conical flask with cover, joined the 70 % ethanol 50 mL, ultrasonic extraction extracted 0.5 h, made up the weight with 70 % ethanol and filtered, then we measured 5 mL filtrate in 10 mL volumetric flask and took supernatant on Millipore filter (0.45 μm) for HPLC analysis.

Preparation of negative reference standard: In order to investigate whether other herbs have an influence to the determination of the samples, it is necessary to be a negative control experiment. According to the prescription and preparation process requirement, we prepared the negative reference standard without baicalin and berberine hydrochloride. Finally, in accordance with the preparation of sample solution method, we prepared the negative reference substance solution.

HPLC conditions: All analysis were performed on an Agilent 1260 series HPLC system (Agilent Technologies, Palo Alto, CA, USA). The separation was performed on an Agilent ZORBAX Eclipse XDB-C_{18} column (5 μm, 4.6 mm × 150 mm). The detection wavelength was set at 280 nm. The mobile phase consisted of acetonitrile (A) and 6 mmol/L KH_2PO_4 solutions (B). The linear gradient was as follows: 0-6 min, 7-21 % A; 6-12 min, 21 % A; 12-27 min, 21-52 % A, at a flow rate of 1 mL/min. The column temperature was maintained at 30 °C and the injection volumn was 5 μL.

RESULTS AND DISCUSSION

Selectivity: Typical chromatograms of the standards, samples and negative reference standard were shown in Fig. 1. A chromatogram of pure standard of baicalin and berberine hydrochloride are shown in Fig. 1(A). These standards are well resolved with relatively high sensitivities at retention time of 9.724 and 18.467 min. A chromatogram of samples of baicalin and berberine hydrochloride are shown in Fig. 1(B). From the Fig. 1(A) and (B), the baicalin and berberine hydrochloride of samples have a same retention time with the reference standards. The negative reference standard are shown in Fig.1 (C) and (D). The Fig. 1(C) is the typical chromatograms without the baicalin and the Fig. 1(D) is the typical chromatograms without berberine hydrochloride. These chromatographic illustrated some impurties of the samples have no interference to baicalin and berberine hydrochloride simultaneous determination.

Linearity, limits of detection and quantification: The linearity, limits of detection (LOD) and quantification (LOQ) of the proposed method were evaluated. Calibration curves of baicalin and berberine hydrochloride were established by plotting the peak area ratios against five different concentrations of the individual analyte. The linear with r^2 of baicalin was 0.9995 and the linearities were evaluated over the concentration range of 1.3625-13.625 μg/mL. The linear with r^2 of berberine hydrochloride was 0.9998 and the linearities were evaluated over the concentration range of 2.525-25.25 μg/mL. The LOQs, defined as the lowest concentration on the

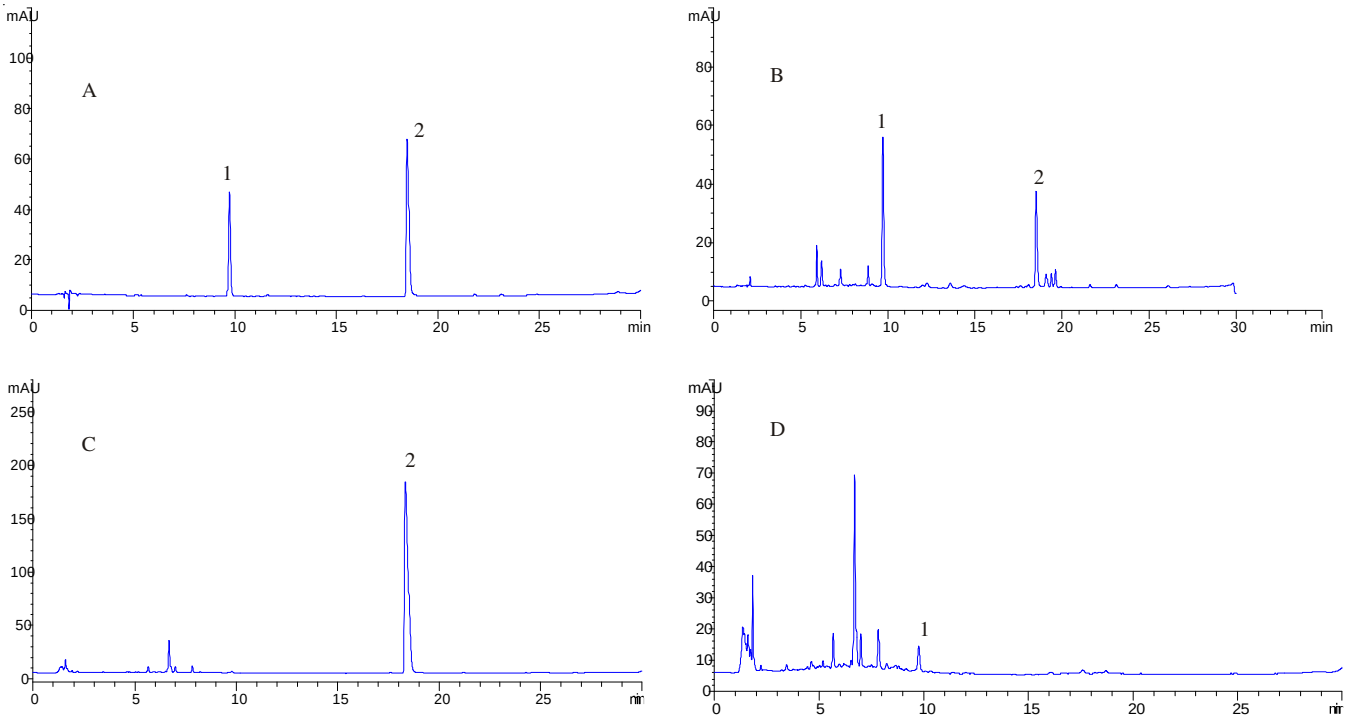

Fig. 1. Representative HPLC chromatograms of standard mixture (A), Fufangkangkui suspension (B), the negative reference standard without baicalin (C) and berberine hydrochloride (D). 1 = baicalin; 2 = berberine hydrochloride

TABLE-1
RESULTS OF REGRESSION ANALYSIS ON CALIBRATION CURVES OF ANALYTES

Analytes	Linearity		LOQ (ng/mL)	LOD (ng/mL)
	Regression equation	R^2		
Baicalin	Y = 7.1121X-10.8125	0.9995	1.62	0.83
Berberine hydrochloride	Y= 3.5125X-9.5687	0.9998	2.86	0.86

calibration curves and determined at a signal-to-noise ratio (S/N > 10), were 1.62 ng/mL for baicalin, 2.86 ng/mL for berberine hydrochloride. LOD, the same procedures were performed with contents of analytes in samples S/N = 3), was 0.83 ng/mL for baicalin, 0.86 ng/mL for berberine hydrochloride, respectively (Table-1).

Precision: The intra-day precision of the method was determined by preparing the standards of baicalin and berberine hydrochloride at three different concentrations and values for each compound were determined by six repeated analyses. Inter-day precision was check with the same concentration as intraday assays and the determination of each compound was repeated day by day during 5 days. The results were given in Table-2. The method was found to be precise with R.S.D. values within 0.98-1.21 % for intra-day assays and 1.06-1.33 % for inter-day assays.

Sample stability: The test of sample stability is the necessary in method validation, the test main explain that the baicalin and berberine hydrochloride do not break down in the prescribed period of time. Working standard solutions were reserved 2, 4, 6, 8, 12 h, then according to the chromatographic conditions determined, the peak area RSD of baicalin and berberine hydrochloride are 0.61 and 0.78 % (n = 6). The results show that the stability of working standard solutions are good.

Recovery: Recovery of the method was estimated by spiking different concentrations of baicalin and berberine hydrochloride into the sample solutions (n = 6). The mean recoveries of baicalin and berberine hydrochloride were 99.4 and 100.6 %, respectively, with the RSDs of < 3 % (Table-3). The recovery data indicate that the sample matrices did not affect the quantitation of the investigated analyte in the samples.

Determination of samples: Three batches of fufang-kangkui suspension from the first people's hospital of bengbu were tested, as shown in Table-4.

In this study, an HPLC methods was developed for the simultaneous determination of baicalin and berberine hydrochloride of fufangkangkui suspension. The method was validated by its linearity, precision, accuracy and reproducibility[7]. It is an important criterion for a high efficiency HPLC condition that the mark peaks have greatly baseline separation with adjacent peaks within a short analysis time as far as possible[8]. There were different HPLC parameters examined and compared, including the following: detection wave lengths of 240, 280 and 260 nm; mobile phases consisting of acetonitrile-6 mmol/L KH_2PO_4 solutions system and methanol-0.1 % phosphoric acid solution system; mobile phase flow rates of 1.2, 1.0 and 0.8 mL/min and column temperatures of 30, 25 and 20 °C[9].

The binary mixtures of the acetonitrile-6 mmol/L KH_2PO_4 solutions system exhibited a more efficient separation ability for the detected compounds than did the methanol-0.1 % phosphoric acid solution system. The 6 mmol/L KH_2PO_4 solutions was added to improve the peak shape[10]. Baicalin and berberine hydrochloride have different chemical structures, properties and absorption spectra and are difficult to baseline resolve

TABLE-2
PRECISION OF PROPOSED HPLC METHOD

Analytes	Concentrations (μg/mL)	n	Intra-day precision RSD (%)	Inter-day precision RSD (%)
Baicalin	13.625	6	10.5	1.13
	5.45	6	1.17	1.26
	1.3625	6	1.21	1.33
Berberine Hydrochloride	25.25	6	0.98	1.06
	10.1	6	1.12	1.20
	2.525	6	1.08	1.11

TABLE-3
RECOVERY TESTS OF BAICALIN AND BERBERINE HYDROCHLORIDE IN FUFANGKANGKUI SUSPENSION

Compound	Amount (μg)	Added (μg)	Measured (μg)	Recovery (%)	Average (%)	RSD (%)
Baicalin	1.9349	1.3625	3.9155	99.70	99.8	0.14
	1.9279	1.3625	3.2895	99.97		
	1.7557	1.3625	3.1059	99.60		
	1.7574	1.3625	3.1201	100.01		
Berberine Hydrochloride	1.7748	1.3625	3.1345	99.91		
	1.7470	1.3625	3.1024	99.77		
	1.7570	2.0200	3.6754	97.31		
	1.7506	2.0200	3.6625	97.13		
	1.5942	2.0200	3.5866	99.24	98.8	1.04
	1.5958	2.0200	3.6122	99.90		
	1.6116	2.0200	3.6135	99.50		
	1.5863	2.0200	3.5866	99.45		

TABLE-4
CONTENTS OF BAICALIN AND BERBERINE HYDROCHLORIDE IN BATCHES OF FUFANGKANGKUI SUSPENSION (mg/g)

No. of batches	Baicalin (mg/g)	Berberine hydrochloride (mg/g)
140302	1.74	1.58
140303	1.76	1.55
140304	1.77	1.56

under isocratic elution. Thus, gradient elution was used in HPLC analysis. The ultimately selected mobile phase system consisted of acetonitrile 6 mmol/L KH_2PO_4 solutions, which provides lower pressure and greater baseline stability. Under these experimental conditions, the retention times of the two components were 0.5 h and the separation was satisfactory[11].

The most suitable flow rate was 1 mL/min. The results also suggested that separation was more efficient when the column temperature was maintained at 30 °C and 240 nm was selected as the chromatogram detection wavelength. Under these conditions, all marker compounds could be detected and showed adequate absorption[12].

Baicalin and berberine hydrochloride are well dissolved in alcohol solution. We compared the 50 % methanol, ethanol, 70 % ethanol as extracting agent in experiment. According to data, there is a highest efficiency when we used 70 % ethanol extract baicalin and berberine hydrochloride from the samples[13]. We adopt the ultrasonic extraction method to extract the sample for 10, 20, 30, 40 and 50 min. As the time extension, the extraction quantity of baicalin and berberine hydrochloride were increasing, but when extracting time was more than the enrichment of the saturation point, it was obvious that the extraction quantity of baicalin and berberine hydrochloride began to decline 0.5 h later, so the extraction time was 0.5 h[14].

Conclusion

A simple, rapid, precise, accurate and reproducible HPLC method using a C_{18} column with UV detection was developed and validated for simultaneous determination of baicalin and berberine hydrochloride in fufangkangkui suspension. This assay is sensitive and reproducible and has been fully validated. It was successfully applied to the quantification of these two compounds in fufangkangkui suspension. Results indicate that this method provides a suitable quality control method for fufangkangkui suspension samples and can be readily utilized for the determination of the major active ingredients present in this complex traditional Chinese medicine (TCM) prescription[15].

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