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Research Article

EVALUATION AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE USE OF BERBERINE FROM *COPTIS TEETA* BARK AS ANTI-CANCER PHYTOCHEMICAL

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ABSTRACT

Objective: A new method of analysis using high-performance liquid reverse chromatography (HPLC) to calculate berberine in *Coptis teeta* bark was created in addition to the validation research.

Methods: Berberine was analyzed with potassium hydrogen phosphate and acetonitrile using the SunFire C18 column at a flow rate of 1.0 ml/min; 5µ (4.6 × 250 mm) was detected at 347 nm.

Results: For precision, selectivity, and linearity, the technique was tested. The quantification of berberine did not conflict with the existence of other chemical components. Between concentrations of 60 and 180 ppm, the calibration curve was linear. The precision ranged from 9.0% to 102.0% and 2% was the relative standard deviation.

Conclusion: Finally, this method of HPLC enhancement is simple, effective and has been used to classify and quantify berberine successfully. This can be useful for the study of different berberine-containing plant extracts and can also be used to measure berberine from formulation to herbal formulation.

Keywords: HPLC, Berberine, *Coptis teeta*, Validation.

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INTRODUCTION

In industrialized societies, about 31.4% of the population, 42–69% in the United States, 71% of Canadians and 90% of the British population consume dietary supplements or natural health products (Vitamins, minerals, amino acids, essential fatty acids, herbal products, traditional Chinese medicines, homeopathic medicines, and probiotics) for treatment, including disposal in urbanized and resource-poor countries, where pretenders sell impure or mixed herbal medicines, where pretenders sell impure or mixed herbal medicines [1,2]. The safety, quality, and efficacy of herbal products is an equally important public health concern. It is possible that the initiation of systematic validation would manage the production of herbal products and medicinal products of mixed or deprived quality, and ultimately ensure their rational use [3]. For quality evaluation and standardization of polyherbal formulations, physicochemical parameters, biochemical analysis, microbiological features, HPLC, and HPTLC fingerprint profiles may be used as marker parameters. HPLC is a fast and accurate technique for individual compound quantification [4].

The "goddess of all healings" is called Ayurveda and is one of the most efficient traditional systems of medicine with many medicinal and healing properties. Individual herbs are not sufficient to achieve the desired therapeutic effect, according to Ayurveda. It offers a stronger therapeutic effect with reduced toxicity when partially configured as a multi-plant composition [1]. Approximately 31.4% of the population in industrial societies, 42–69% in the United States, 71% in Canada, and 90% in the United Kingdom consume dietary supplements or natural health products (Vitamins, minerals, amino acids, essential fatty acids, herbal products, traditional Chinese food medicines, homeopathic medicines, and probiotics) for therapeutic purposes. In developed nations, where fake herbal medicines are marketed and in countries without money, the quality of herbal products is a major public health issue. The implementation of scientific verification should regulate the production of, and eventually ensure the fair use of, low-quality herbal or herbal products [3]. As parameter markers for quality evaluation and standardization of pilocarpine formulations, physicochemical parameters, biochemical analysis, microbiological properties, HPLC, and HPTLC fingerprint profiles can be used. HPLC is a rapid and precise process for the quantification of individual compounds [4].

Coptis teeta Wall is a perennial plant belonging to the family Ranunculaceae. It is rich in alkaloids such as lignans, phenylpropanoids, flavonoids, phenolic acids, carbohydrates, and steroids such as berberine, palmatine, jatrorrhizine, coptisine, columbamine and epiberine, and several secondary metabolites [5,6]. Over the past decade, extensive research has shown that berberine has a wide range of pharmacological activities, including antimicrobial and antibacterial activity, diarrhea, antihypertensive activity, rhythmic activity, antihypertensive activity, anti-inflammatory activity, antihypertensive activity, depressive activity, antioxidant activity, and antidiabetic activity [7,8].

In this study, our objective is to use RP-HPLC-UV-DAD to differentiate, identify and measure the single natural anticancer drugs, i.e., berberine in *Coptis teeta* plant extract. Furthermore, validated was the HPLC-DAD process for measuring these drugs. The validation of chromatographic recognition of berberine may contribute to the process of equivalence between herbal extracts and polyherbal formulation and drug expansion.

METHODS

Chemicals

HPLC-grade solvents such as acetonitrile, methanol, orthophosphoric acid, and water were obtained from Merck Ltd. Bangalore India. Standards from Natural Remedies Ltd. Bangalore India were purchased (Table 1).

Plant material

The *Coptis teeta* fresh bark was acquired from the local market, Pune District, Maharashtra State, India. Department of Pharmacognosy

Alard College of Pharmacy Pune confirmed their identity and authentication by correlating their morphological and microscopic characters with those given in literature. In the shade, the fresh bark was dried, finely powdered, and the powder was passed through 80 mesh sieves and deposited at room temperature (30±2°C) in airtight containers.

Extract preparation

The powdered root was processed for 72 h at 80°C with a water-alcohol mixture (1:1). The extract was collected, filtered and vacuum dried with a rotary vacuum evaporator after full extraction (Buchi). The extract has been stored for further use in an airtight glass bottle. Validation of the alcoholic extract method was carried out.

Preparation of standard solution

Berberine 10 mg was precisely weighed and transferred into a volumetric flask of 25 mL. 20 mL of diluent was added and sonicated for 15 min in an ultrasonic water bath. The solution was cooled and diluent volume was produced up to the mark.

Preparation of test solution

Approximately 20 mg of plant extract was consumed in a 100 mL volumetric flask. 70 mL of diluent was added and then echoed for 20 min in an ultrasonic water bath. The solution was cooled and diluent volume was produced. It was then filtered with a 0.45 μ syringe filter. The test solution was used as the resulting solution.

Chromatographic conditions for HPLC

A Waters 2695 Alliance system with a 2996 photodiode array detector (PDA) and 2489 UV/Visible detector was used to conduct HPLC (UV). On a reverse-phase, 250 × 4.6 mm, 5-μm, Sunfire C18 column, the standard was resolved as Berberine. A mobile phase of 50 millimoles of potassium di-hydrogen phosphate in water was prepared and pH 2.5 was modified with diluted OPA (solvent-A) and acetonitrile (100%) (Solvent-B). The applied gradient software is defined in Table 2. The flow rate of the mobile phase was held at 1 ml/min. The column was inundated for 30 min through the preliminary mobile process prior to the first injection. The column temperature has been held at 30°C. The volume of the injection was determined to remain at 10 μL. By optimizing wavelength, the PDA was set to provide the best response for two peaks at 347 nm to obtain the chromatogram. By comparing the retention time and spectrum obtained from the sample and standard solutions, the standard Berberine was established. The current attempt was made in an air-conditioned room maintained at a temperature of 25°C [9-11].

Preparation of calibration graph

From 50% to 150% of the working concentration level for Berberine, the linearity of peak area response for Berberine was identified. Seven different known concentrations of the stock solution of Berberine were diluted. The concentration map (as X-value) against the region (as Y-value) was planned.

VALIDATION OF HPLC METHOD

In terms of specificity, precision, accuracy, and robustness, the projected HPLC technique was validated as per the guidelines of the International Conference on Harmonization (ICH) [12-15].

Specificity

The specificity of the technique was analyzed by estimating the peak purity of berberine using the Waters empower software program and diode array detector moreover shows of purity angle, purity threshold, and purity flag.

Table 1: Standard used for validation studies

S. No.	Name of standard	Batch No	Potency (%)
1.	Berberine	T12H035	93.80

Precision

Precision was analyzed in terms of device precision, precision of the procedure, and intermediate precision.

System precision

System precision was performed by six repeat injections from the same standard vial and was expressed in terms of tailing, plate count, and resolution percent relative standard deviation (percent RSD).

Method precision

The sample was examined six times by the aforementioned system. In terms of percent RSD, the percent assay for Berberine was articulated.

Intermediate precision

Intermediate precision was performed by various analysts on various systems, one with a 2996 PDA Waters e2695 Alliance system and the other with a 2489 ultraviolet (UV) detector by scrutinizing six distinct extract samples and was recorded in terms of percent RSD.

Recovery studies

From recovery research, the accuracy of the procedure was determined by adding an identified quantity of standard to the pre-analyzed sample at the 80%, 100%, and 120% stage, accompanied by a duplicate quantitative study of the proposed technique.

Robustness

The technique's robustness was determined by a slight divergence in the parameters of the system. Variations in column chemistry, wavelength, column temperature, flow rate, and mobile phase gradient were the parameters selected. The retention time of berberine was determined and a percentage of RSD was observed using parameters of device suitability.

To verify the content of berberine according to the method defined under chromatographic conditions by HPLC, herbal extract was analyzed. All inquiries were replicated three times, with implications expressed in mean±SD.

RESULTS AND DISCUSSION

In the HPLC technique, the mobile phase ratio was optimized by measuring various solvent compositions of unreliable polarity, column chemistry, column temperature, and mobile phase pH, and the finest fallout was obtained using the current technique, which produces highly proportionate peaks with good resolution between each standard and other peaks (Fig. 1). In addition, at this wavelength analyte, the scanning wavelength selected was 226 nm to provide similar performance, showing optimal response. With a holding time of 11.4 min, Berberine was acceptably resolved.

The calibration graph showed a working concentration level of 50–150% for berberine with appropriate correlation coefficients of 0.9990 and 0.9992 for berberine (59–184 μg/mL) (Table 3). Fig. 2 shows the graph for each standard.

In system precision, method precision, and intermediate precision values are defined against sample application and peak area scanning and are expressed in terms of percent RSD. Percent RSD values were found to be 1.06% for Berberine for machine precision. The precision

Table 2: Details of Gradient program

Time (min)	Flow (mL/min)	% solvent A	% solvent B
0	1.0	80	20
25	1.0	40	60
30	1.0	50	50
35	1.0	50	50
40	1.0	80	20
45	1.0	80	20

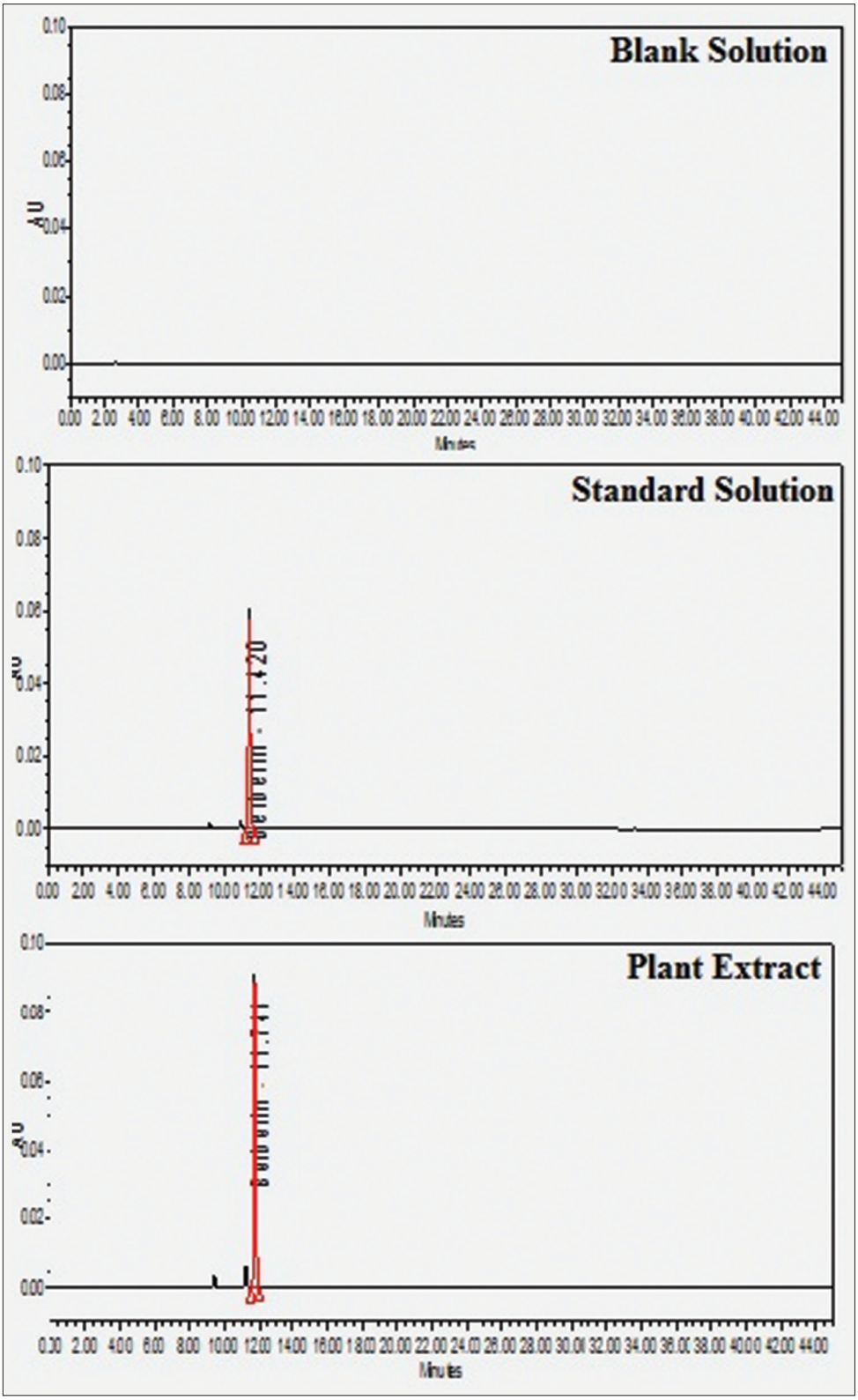

Fig. 1: Chromatograms for blank, standard, and sample

of the method was achieved and the percent RSD value for Berberine was found to be 1.02%. Percent RSD values among the two analysts for intermediate precision were found to be 1.39% for Berberine. The percent RSD values for the values of system precision, method precision, and intermediate precision showed that the proposed methodology offers a reasonable degree of system precision, method precision, and intermediate precision.

Comparing their respective spectra at peak start, peak apex, and peak end positions of the spot from standard and extracts, the peak purity of each analyte was evaluated. In Table 4, the purity angle and purity threshold values are given.

Via robustness, the method given was optimized. For each parameter, the peak area for each analyte was determined and less than 2% percent

Table 3: Linearity of berberine

% Level	Conc. of berberine (ppm)	Average peak area of berberine
50	59.4	207133
60	75.6	264344
70	97.2	346647
100	118.8	426788
120	140.4	494727
140	162.0	585161
150	183.6	659179
R^2	0.9994	
Slope of Regression line	3645	
y-intercept	9649	

Table 4: Specificity of berberine

Sr. No.	Sample name	Analyte name	Purity flag	Specificity
1.	*Coptis teeta* Extract	Berberin	No	Specific
2.	Standard	Berberin	No	Specific
3.	Blank	No Peak	-	-

Table 5: Robustness for berberine

Robustness parameter		% RSD	Peak tailing	Theoretical plates	Remark
Wavelength (nm)	342	0.55	1.134	44410	Pass
	347	0.67	1.120	44646	Pass
	352	0.54	1.134	44411	Pass
Temperature (°C)	20	0.57	1.15	43364	Pass
	25	0.67	1.12	44646	Pass
	30	0.39	1.11	41507	Pass
Flow (mL/min)	0.9	1.66	1.058	49428	Pass
	1.0	0.67	1.116	44645	Pass
	1.1	0.32	1.122	39620	Pass

Table 6: Recovery for berberine

Analyte	Recovery level	% Recovery	Average % recovery
Berberine	80% - 1	100.64	100.28
	80% - 2	104.78	
	80% - 3	95.41	
	100% - 1	96.15	103.40
	100% - 2	107.42	
	100% - 3	106.63	
	120% - 1	102.76	101.06
	120% - 2	102.31	
	120% - 3	98.12	

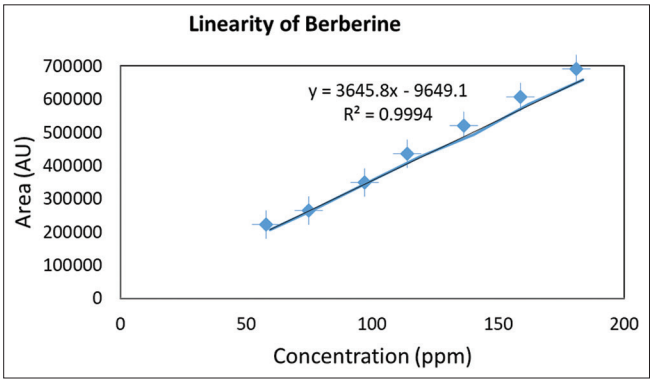

Fig. 2: Linearity graphs for standard

of RSD was found. The percent RSD values as shown in Table 5 indicate improved method robustness.

The recovery analysis was performed at 80%, 100%, and 120% of working concentration by spiking accepted amounts of standards into placebo solution. The overall percentage of recovery was found to be 101% for Berberine (Table 6).

CONCLUSION

The current investigation culminated in the development of a Berberine RP-HPLC-UV-DAD analysis method that was validated in terms of linearity, consistency, precision, specificity, suitability, and robustness of the device. In addition to its novelty for the determination of berberine at single wavelength, the presented method is sufficiently fast, simple, and sensitive as well as accurate and precise in accordance with ICH guidelines. The assay of the active ingredients did not interact with the excipients in the other plant chemicals. For routine analysis of berberine, the proposed analytical method is therefore recommended, either as such or in different dosage types. Moreover, in many developing countries or field stations, where specialized analytical equipment is not usable, the approach can be applied.

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AUTHORS' CONTRIBUTION

The involvement of Dr. Madhuri Chondhe and Miss Swapnali Hande included the selection of samples, the design and performance of laboratory work, the interpretation of findings, and the preparation of the report. Contributions included data analysis and identification of the compounds by Dr. Madhuri Chondhe, Miss Swapnali Hande and Dr. Sonia Singh. Each author interpreted and permitted the submission of the last manuscript.

CONFLICTS OF INTEREST

No conflicts of interest are declared by the authors.

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None.

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