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High performance liquid chromatographic Assessment of berberine in Methanol Extract of diverse parts of *Tinospora cordifolia*

B.C. Akhilraj[1], J. Suresh[2]*, K. Rajamani[3] M. Kumar[4] and R. Gnanam[5]

[1]Department of Plantation, Spices, Medicinal and Aromatic Crops, HC & RI, TNAU, T.N., India
[2]Coconut Research Station, TNAU, Aliyar Nagar, Pollachi 642 101, T.N., India
[3]Department of* Floriculture *and Landscape Architecture, TNAU, Coimbatore, T.N., India
[4]ICAR-KVK, TNAU, Tindivanam, T.N., India
[5]Department of Bio Informatics, CPMB&B, TNAU, Coimbatore, T.N., India

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ABSTRACT

In the current work, a straight forward, affordable, reliable, repeatable, selective, and accurate high-performance liquid chromatography (HPLC) approach for berberine measurement in both a 60% methanolic extract of *Tinospora cordifolia* and a commercial formulation was developed and validated. The berberine peak, which has a retention time of 9.52±1.03 min, is crisp and well-defined in the acetonitrile-water (25:75%, v/v) mobile phase. Berberine is estimated using HPLC at a wavelength of 266 nm and a flow rate of 1.0 ml/min. The calibration curve's results from linear regression analysis demonstrates a strong linear association with a correlation coefficient of 0.997 in the concentration range of 0.5 to 50 g/ml. y=29716x-4417.4 is the equation for the linear regression. The thresholds for quantification and detection are 0.18 and 0.55 g/ml, respectively. According to ICH criteria, the proposed technique has been verified for accuracy, precision, repeatability, and robustness. For the purpose of estimating berberine in methanolic extract and formulation, the proposed approach is used, which has a high degree of precision and accuracy. The approach is exact, repeatable, selective, and accurate for estimating berberine, according to statistical analysis.

Key words: *HPLC, Berberine, Tinospora cordifolia, Methanolic extract*

Introduction

Thousands of years, millions of people have utilised herbal remedies all throughout the world. Eighty per cent of the world's population, particularly in underdeveloped nations, depends heavily on herbal remedies for their health. According to the World Health Organization's (WHO) Traditional Medicines Programme, due to their low toxicity and known pharmacological activity, nearly 80% of the world's population used phyto-products. Growing demand for herbal treatments is shown by the trade in plant-based pharmaceuticals and raw materials, which has had annual growth rates between 5 and 15%. Due to the considerable unpredictability of the associated chemical components, quality control and assurance still pose a difficulty. The use of chromatographic and spectral fingerprint analyses has greatly improved our ability to ensure the quality of herbal medicines (Kumar *et al*., 2000; Bairy *et al*., 2002).

The menispermaceae family includes the big, gla-

([2*]Prof. and Head, [3]Prof. and Head, [4]Prof. (PB&G) & Programme Coordinator, [5]Prof. and Head)

brous, deciduous climbing shrub known as guduchi (*Tinospora cordifolia*). It may reach an altitude of 1200m and is found all throughout the tropical Indian subcontinent, Sri Lanka, and China. *T. cordifolia* has a relatively succulent stem with long filiform aerial roots that are made of flesh. The bark is deeply left rosette-like and ranges in colour from creamy white to grey. The leaves are cordate and membranous. The blooms are tiny, greenish yellow or yellow blossoms (Jagetia and Rao, 2007)

Tinospora cordifolia, sometimes referred to as Amrita (Guduchi) in Sanskrit and Shindilkodi in Tamil, is a plant that is frequently used in ayurveda and traditional medicine. The propensity of *Tinospora cordifolia's* aerial portions, stem, leaf, roots and fruits to contain a vast number of chemicals has led to the plant being given the name Amrita, which means "to provide youth, vigour, and life to the consumer" (Manjrekar *et al*., 2000; Prince and Menon, 2000). In the "Rasayanas" of the Ayurvedic medical system, guduchi is frequently used to boost the immune system and increase body resistance to illnesses. In more recent years, reports of the immunomodulatory properties, antineoplastic activities, and hypoglycemic activity of *T. cordifolia* have been made. This plant is currently used in modern medicine to treat anaemia, viral hepatitis, urinary diseases, dyspepsia, dysentery, dyspepsia, and general weakness (Prince and Menon 2001, Singh *et al*., 2010; Jagetia and Rao, 2006).

No analytical techniques have been described for the measurement of berberine in the plant, its extract, and in formulations, according to a thorough review of the literature. The purpose of the current study was to develop an easy, affordable, selective, accurate, repeatable, and reliable high-performance liquid chromatographic (HPLC) method for the estimation of berberine in the natural plant, its extracts, and commercial tablet formulation using UV detection. Berberine extracts have been claimed to have Hepatoprotective and anti-inflammatory effects. Because it is simple to learn and use and is not constrained by the volatility or stability of the sample chemicals, HPLC is a common approach for the analysis of herbal medicines. In the current investigation, the quantification of berberine was carried out using acetonitrile and water as the mobile phase. A sensitive technique was devised and validated in accordance with ICH criteria.

Materials and Methods

The stem, leaf and root of *Tinospora cordifolia* was collected from department of medicinal and aromatic garden, Tamil Nadu agricultural university, Coimbatore (11.0122° N, 76.9354° E), Tamil Nadu.

Tinospora cordifolia extract preparation

Here we're used Microwave assisted extraction (MAE) for the methanol extraction. The different plant parts *viz*., stem, leaf, root, seed, fruit of *T. cordifolia* freshly collected and shade dried for one week. Well dried parts were powdered and allowed to pass through SS sieve (20 mesh). The medication was transported in a 20 gramme powdered form to a 500 ml conical flask. A mixture of water and 200 millilitres of ethanol at 80% (v/v) was added. The mixture was well mixed and let to stand for a while so that the medication could absorb the solvent. The extraction temperature and irradiation power were both set at 480 W for 3 minutes. The conical flask was removed from the oven once the extraction process was finished (Sajitha *et al.,* 2015). To get the crude extract, which is kept in a dessicator for later use, the crude extract was distilled out at a lower temperature and at a lower pressure in a rotating flash evaporator. Each and every chemical and reagent utilised was of an analytical and HPLC calibre. From Sigma, berberine with >99.0% purity was acquired

Preparation of standard solution

9.1 mg of the chemical berberine, $C_{20}H_{18}C_lNO_4$ (Molecular Weight: 371.81), was weighed and dissolved in 10 ml; 1 ml of this solution was then diluted to 10 mL, yielding 54.49 g/ml equivalent of standard berberine. To prepare a standard stock solution of berberine 50 g/ml equivalent, the 5 ml of the previously mentioned solution was diluted to 5.449 ml with fresh solvent using a micropipette. These dif-

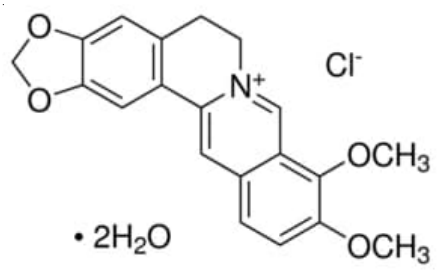


Fig. 1. The chemical structure of berberine (2D)

ferent concentrations for the calibration curve were then obtained by further diluting the standard stock solution. The standard and sample were filtered through 0.2 μm syringe filter and the filtrate was transferred to HPLC vial.

Equipment and Conditions

Used was a Shimadzu type HPLC (Kyoto, Japan) with quad LC-10A VP pumps, a Shimadzu SPD-10AVP column oven with an adjustable wavelength programmable UV/VIS detector, a Shimadzu SCL 10AVP system controller, a Shimadzu Rheodyne injector with a 20-L loop, and Class-VP 5.032 software. A reverse phase C18 Zorax RP-HPLC column (250 4.6 mm, 5 m) was the chromatographic column that was utilised. The HPLC apparatus and column were maintained at room temperature. Acetonitrile and water were used as the mobile phase, with a flow rate of 1.0 ml/min. A 210 nm wavelength was used to evaluate the elute after a 20 L infusion.

Method development

For the development of an appropriate HPLC technique for the quantification of berberine in 60% methanolic extract and commercial formulations, various solvent systems were tested. Methanol-water (25:75%), acetonitrile-water (25:75%), methanol-water (50:50%), acetonitrile-water (50:50%), and acetonitrile-water (75:25%) were the mobile phases tested for these applications. Cost, assay sensitivity, and the amount of time needed for the analysis were taken into account while determining the solvent system's appropriateness.

Calibration curve of berberine

For the creation of the calibration curve from the produced stock solution, several concentrations (0.5–50 g/ml) were made. For column standardisation, the mobile phase was given at a rate of 1.0 ml/min following filtering via a 0.45-m membrane filter, and baseline was constantly measured during the procedure. The 266 nm detecting wavelength was used. Areas under the peaks for each of the prepared dilutions were recorded after they were each serially injected. The stability of the medication in solution during analysis was assessed by multiple sample analyses throughout the experiment on the same day as well as after 48 hours of storage of the drug solution in the laboratory and the refrigerator.

Method validation

Linearity

The linearity of berberine was tested across the concentration range of 0.5 to 50 g/m. For linearity, a graph was drawn between concentration and area beneath the peak.

Accuracy as recovery

The usual addition procedure was used to assess accuracy. The berberine (10 g/ml) preanalyzed samples were spiked with an additional 0, 50, 100, and 150% of the standard berberine, and the mixes were reanalysed using the suggested technique. At each concentration level, the percentage (%) sample recovery, percentage relative standard deviation (% RSD), and standard error were computed.

Precision

Repeatability and intermediate precision were measured in terms of precision. Inter-day variation was used to assess intermediate precision for the measurement of berberine at four distinct concentration levels of 5, 10, 20, and 40 g/mL in triplicates, while intra-day variation was used to determine repeatability of sample application.

Table 1. Linear Regression Data for the Calibration Curve (n = 3)

Linearity range (μg/ml)	0.5–50
Regression equation	y = 29716x – 4417.40
Correlation coefficient	0.997
Slope ± SD	29716 ± 92.41
Intercept ± SD	4417.40 ± 31.85
Slope without intercept ± SD	29590 ± 90.74
Standard error of slope	53.38
Standard error of intercept	18.38
95% confidence interval of slope	29486.30 – 29945.69
95% confidence interval of intercept	4338.31 – 4496.48

Reproducibility

By getting accuracy on a separate instrument, which was then assessed by a different individual in a different laboratory, the method's reproducibility was examined. At four distinct concentration levels, 5, 10, 20, and 40 g/mL in triplicates, both intra-day and inter-day precision were calculated.

Limits of quantification and detection

By using the standard deviation (S y/x) technique, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated. A blank sample was injected into the chromatograph in triplicate to determine the LOD and LOQ, and the peak area of this blank was then measured. LOD = 3.3 S y/x /S and LOQ = 10x S y/x /S, where S y/x is the standard deviation of the blank response and S is the slope of the calibration curve, were calculated using the slope of the calibration curve and S y/x of the blank sample.

Robustness

For the purpose of determining the amount of berberine, robustness was used to assess the impact of a minor but intentional alteration in the chromatographic settings. The method's robustness was tested by varying the mobile phase's flow rate (1.1 and 0.9 ml/min) and acetonitrile concentration (23 and 27%).

Quantification of Berberine

Chromatograms of the test samples were produced under the identical circumstances as those used to produce standard berberine after being injected. Recording the peak size of the peak that corresponded to the Rt of standard berberine allowed for the calculation of its content using the regression equation derived from the calibration curve.

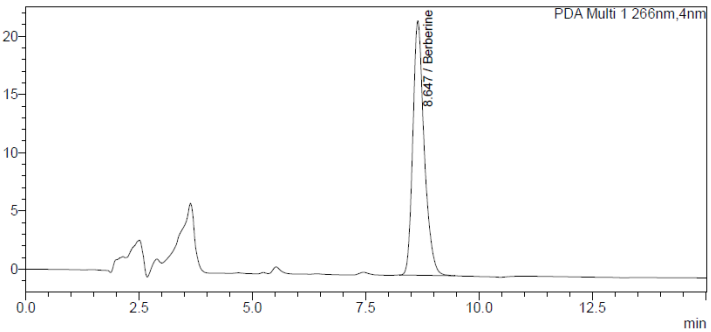
Results and Discussion

Method development

For the purpose of creating a practical and precise HPLC technique for berberine measurement, the mobile phase composition was improved. Methanol-water (25:75%), acetonitrile-water (25:75%), methanol-water (50:50%), acetonitrile-water (50:50%), and acetonitrile-water (75:25%) were the mobile phases tested for this purpose. With the lowest Rt (8.64 min) and sharpest, well-defined peak, the chromatogram (Figure 2) produced by the acetonitrile-water (25:75%) solvent system was determined to have extremely high symmetry (1.15). As a result, the ratio of 25% acetonitrile to 75% water was chosen as the mobile phase. The medication remained stable for 48 hours while being stored at room temperature and in an acetonitrile-water (25:75%) combination.

Calibration Curve

In the concentration (g/ml) range of 0.5 to 50 g/ml, the calibration curve area was determined to be linear. Calculations in statistics were performed at the 5% level of significance. To compare the outcomes, a one-way analysis of variance (ANOVA) test was used. As shown in Table I, the calibration curve's linear regression data demonstrated a satisfactory linear relationship with regard to peak area across the concentration ranges of 0.5–50 g/ml. Table I shows that the correlation coefficient value (R2) was 0.997, which is extremely significant (p 0.05). y =



<Peak Table>

PDA Ch1 266nm

Peak#	Name	Ret. Time	Area	Height	Conc.	Unit
1	Berberine	8.647	381993	21851	5.000	mg/L
Total			381993	21851		

Fig. 2. Berberine HPLC chromatogram with Rt at 8.647 min in acetonitrile and water (25:75% v/v)

29716x - 4417.40 was the equation for the linear regression. The slope of the standard curves did not change significantly (p 0.05). It was discovered that the retention time and asymmetry factor were, respectively, 8.674± 1.11 min and 1.19 ± 0.12 min.

Validation of the method

Linearity

The range of linearity for berberine solutions was determined to be 0.5 to 50 g/ml.

y = 29716x - 4417.4 was the regression equation, and the correlation coefficient was 0.997.

Accuracy as recovery

Recovery analysis, which allowed for a recovery of 99.21–99.82% after adding an extra standard drug solution to the previously examined test solution, was used to determine the correctness of the suggested approach. The percentage RSD values were found to be between 1.42 and 1.97. These outcomes demonstrated how accurate the suggested approach was.

Precision

Precision was taken into account at two levels of ICH recommendations (i.e., repeatability and intermediate precision). For the assessment of berberine at four distinct concentration levels of 5, 10, 20, and 40 g/ml in triplicates, intra-day variation was used to determine repeatability of sample application, while inter-day variation was used to determine intermediate precision. Table 2 displays the repeatability and intermediate precision results, which were reported as a percentage of RSD. The proposed procedure can be repeated because of the low values of % RSD.

Reproducibility

The method's reproducibility was evaluated by confirming its accuracy in a separate lab using different tools and being examined by a different individual under the same circumstances. In laboratories, both intra-day and inter-day accuracy were looked at. The repeatability of the procedure is demonstrated by the lack of significant changes in the % RSD values of intra-day and inter-day accuracy (Table 3).

LOD and LOQ

The standard deviation method described in the Experimental section was used to calculate the LOD and LOQ of the proposed method, and the results showed that they were 0.18 and 0.55 g/ml, respectively. This showed that the proposed method can be used successfully over a wide range for berberine detection and quantification. The sensitivity of the suggested approach is indicated by the low values of LOD and LOQ.

Robustness of the method

By altering the mobile phase's composition and flow rate, there was no discernible difference in the retention time of berberine. As seen in Tables 4 and 5, a

Table 2. Accuracy of the suggested method

Conc. (µg/ml)	Repeatability (Intra-day precision)			Intermediate precision (Inter-day)		
	Mean area ± SD (n = 3)	SE	% RSD	Mean area ± SD (n = 3)	SE	%RSD
5	152972.54	269.77	0.30	152405.38	282.59	0.32
10	285856.98	1724.89	1.04	285104.62	1746.73	1.06
20	604272.21	4354.11	1.24	602254.11	4529.75	1.30
40	1121858.25	1448.41	0.22	1121085.14	1686.76	0.26

Table 3. Reproducibility of the Proposed Method

Conc. (µg/ml)	Repeatability (Intra-day precision)			Intermediate precision (Inter-day)		
	Mean area ± SD (n = 3)	SE	%RSD	Mean area ± SD (n = 3)	SE	%RSD
5	145248.44	505.43	0.60	141254.23	568.37	0.69
10	265432.11	3010.67	1.96	262154.89	2958.62	1.95
20	601451.33	4873.68	1.40	598954.41	5280.46	1.52
40	1116589.55	2833.58	0.43	1112154.12	3116.88	0.48

Table 4. Modifying the Mobile Phase to Increase the Method's Robustness

Conc. (µg/ml)	Mobile phase composition acetonitrile–water			Mean Rt ± SD (min)	% RSD
	Original	Used	Level		
10	25:75	23:77	-2	8.614	1.74
		25:75	0	8.674	1.25
		27:73	+2	8.772	1.82

Table 5. The Method's Robustness by Changing the Flow Rate

Conc. (µg/ml)	Flow rate (ml/min)			Mean Rt ± SD (min)	%RSD
	Original	Used	Level		
10	1.0	0.9	-0.10	8.803	1.66
		1.0	0	8.674	1.23
		1.1	+0.10	8.572	1.75

low result for the% RSD suggested the robustness of the approach.

Quantification of berberine in *T. cordifolia* methanolic extracts

By comparing the retention durations obtained from the peaks with those of the standard, the peaks of berberine from the sample solution were identified. The 60% methanolic extract of *T. cordifolia* was prepared using the same HPLC parameters as the standard berberine estimate (Rt8.674 min). It was shown that *T. cordifolia* extract from the stem zone had the highest concentration of berberine, followed by samples from the root and leaf areas. Using the regression equation, berberine was measured in *T. cordifolia* extract, and levels of 0.70%, 0.20%, and 0.10%w/w were observed in the stem, root and leaf samples, respectively.

Conclusion

Both methanolic extracts and commercial formulations of berberine can be quantified using the suggested verified HPLC technique. Therefore, this method can be explored for standardisation and quality control of raw materials like stem, root and leafof *T. cordifolia*. It can be used successfully for the routine analysis of berberine in diverse parts of the plant without any interference.

Conflict of Interest

There are no conflicts of interest, according to the authors.

References

Bairy, K.L., Roopa, K., Malini, S. and Rao, C.M. 2002. Protective effect of *Tinosporacordifolia* on experimentally induced gastric ulcers in rats. *J. Nat. Remedies*. 2 : 49-53.

Jagetia, G.C. and Rao, S.K. 2006. Evaluation of cytotoxic effects of dichloromethane extract of guduchi (*Tinospora cordifolia* Miers ex Hook F & THOMS) on cultured HeLa cells. *Evidence-Based Complementary and Alternative Medicine*. 3(2): 267-272.

Jagetia, Ganesh Chandra and Shaival Kamalaksha Rao, 2007. Evaluation of the antineoplastic activity of guduchi (*Tinospora cordifoli*a) in Ehrlich ascites carcinoma bearing mice. *Biological and Pharmaceutical Bulletin*. 29 (3): 460-466.

Kumar, S., Verma, N.S., Pande, D. and Srivastava, P. S. 2000. In vitro regeneration and screening of berberine in *Tinospora cordifolia. J. Med. Arom. Plant Sci.* 22: 61–66.

Manjrekar, P.N., Jolly, C.I. and Narayanan, S. 2000. Comparative studies of the immunomodulatory activity of *Tinospora cordifolia* and *Tinosporasinensis*. *Fitoterapia*. 71(3) : 254-257.

Prince, P., Stanely Mainzen and Menon, V.P. 2001. Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives.* 15(3): 213-218.

Satija, S., Bansal, P., Dureja, H. and Garg, M. 2015. Microwave assisted extraction of *Tinospora cordifolia* and optimization through central composite design. *Journal of Biological Sciences.* 15(3): 106.

Singh, S., Srivastava, R. and Choudhary, S. 2010. Antifungal and HPLC analysis of the crude extracts of *Acoruscalamus, Tinospora cordifolia* and *Celestrus paniculatus*. *Journal of Agricultural Technology*. 6(1) : 149-158.