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Development and Validation of RP-HPLC Method for Quantification of Berberine in Ethanol Fraction of Methanol Extract and Developed Formulation of *Tinospora cordifolia*

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ABSTRACT

Simple and precise RP-HPLC method was developed for quantification of berberine in ethanol fraction of methanol extract of *Tinospora cordifolia* and its formulation. In this, separation was achieved on a HPLC System (Shimadzu) Luna, C18 column. As mobile Phase acetonitrile and water (40:60) were used with a flow rate of 1ml/min and maximum absorbance was found at 266nm. In this we obtained calibration curve in the range of 20 - 640 mg/mL. The slope was 68734, intercept was 20563 and correlation coefficient was found to be 0.999. The limit of detection was 0.8 and limit of quantification was 1.7. For berberine the method was validated according to ICH guidelines. Method was highly precise and accurate because it shows low relative standard deviation as well as good % recovery values. Quantity of berberine was found was found to be 282.3 ppm. In tablet of 325mg.

Keywords: Tinospora cordifolia*, Berberine*, RP-HPLC, Formulation of the solid oral dosage form.**,** Validation.

INTRODUCTION

T. cordifolia belonging to Menispermaceae family, is also known as Guduchi*,* Amrita and Giloy. It showes a wide range of pharmacological effects such as general tonic, analgesic, hypoglycaemic and immunomodulatory agent[1-8]. It contains alkaloids, glycosides, terpenoids, steroids, bitters and carbohydrates. It contains berberine, an isoqunoline alkaloid, present in roots, rhizomes and stem bark of plants. Berberine extracts have been reported to possess hepatoprotective and anti-inflammatory actions.[9-14] Besides its significant antimicrobial activity, berberine is also effective (200 mg/kg/day) in ameliorating diabetic nephropathy in rats[15-16]. In some research papers analysis of berberine in Fork capsule and marketed tablet, were shown by using LC with UV detection. HPLC method development and validation for the berberine from *Berberis aristata* andStandardization and formulation of *Tinospora Cordifolia* Tablet. for polyherbal formulation HPLC conditions were also describe.. HPLC method

determination of berberine in *Coscinium fenestratum* plant extract and RP-HPLC determination of berberine in homoeophatic formulation also reported[17-20]. In present study quantification of berberine was done by using acetonitrile and water as mobile phase and sensitive method was developed and validated as per ICH guidelines.

MATERIAL AND METHOD

All the chemical and reagents used were of HPLC and analytical grade. Berberine, >99.0% purity was purchased from Sigma, (USA).

T. cordifolia stems were collected from the Bangrasia village (10 km from the Bhopal, and authenticated by the Botany Department Saifia Science college, Bhopal (M.P) and voucher specimens were deposited (420/ Bot./saf./14). Shade dried powder of stems of *T. cordifolia* were subjected for successive solvent extraction using Soxhlation method. On the basis of polarity index (PI), fractionation of methanol extract was done using benzene, ethyl acetate and ethanol by silica gel 60-80 mesh packed chromatographic column. The solvents were removed by distillation and last traces of solvents were removed under vacuum.

Development of formulation. cordifolia tablets were prepared by using spray dried powder of ethanol fraction of methanol extract by direct compression method using microcrystalline cellulose (MCC) and Pregelatinized starch (PGS). Talc used as lubricant and diluents, magnesium stearate used as glidant. The weight of prepared tablet was 325 mg.

Table 1: Chromatographic Parameters for berberin

Column name	Hypersil ODS 5 microns (4.6 × 250mm, 5 µm)
Flow rate	1.0 ml per minute
Injection volume	10 µL
Detector	UV at 266 nm using SPD–M10 AVP photodiode array detector
Temperature	30o C
Mobile phase	Acetonitrile : water (40:60)

Instrumentation and chromatographic condition

During RP-HPLC method development, chromatographic conditions adopted are summarized as table no 1.

Praration of mobile Phase

To 40 part of acetonitrile and 60 part of water was mixed to get one liter of mobile phase and filtered through 0.22 µm nylon membrane vaccum filteration and degassed by sonication.

Sample preparation for HPLC

Preparation of Standard solutions

Accurately weighed 10 mg of berberine was placed into a 10 ml volumetric flask dissolved in ethanol (stock solution).

Preparation of stock solution of ethanol fraction of methanol extract

100 mg of dry powdered sample (ethanol fraction of methanol extract) of *T. cordifolia* was dissolved in 80 ml of ethanol, then sonicated for 10 min volume was made up to 100 ml with ethanol after that pass it through 0.22 µm millipore filters.

Preparation of sample solution of developed tablet

Ten tablets were taken and powdered separately in pestle and mortar and per tablet weight was taken and dissolved in small quantity of ethanol then volume was made up to 100 ml. The standard and sample were filtered through 0.15 µm filter paper and the filtrate was transferred to HPLC vial and placed inside the HPLC auto sampler chamber. The system was standardized for the HPLC instrument and the analysis was accomplished.

Table 2 : Concentration and peak area of Berberine

S. No.	Concentration (µg/mL)	Peak area
1	20	1498012
2	40	2821424
3	80	5728468
4	160	11390753
5	320	22287245
6	640	44112785

Method validation

Method validation was done according to ICH guideline on different parameters like specificity, range[21-22].

Specificity study

To overcome the excipient effect specificity study was done. For this chromatogram was taken by appropriate dilution and quantities of drug were determined and compared withs blank solution.

Linearity and Range

The linearity is directly proportional to the concentration of the analyte in the sample. Stock solution of berberine was suitably diluted with ethanol to get concentrations in the linear range 20 to 640mg/ml for HPLC analysis .The slope, intercept and correlation co-efficient values were determined. Dilutions were injected and chromatograms were recorded. Linearity graphs with correlation co-efficient for berberine were plotted, using concentrations on X-axis and the respective peak areas on Y-axis Table 2, Table 3 and Figure 1.

Limit of detection and Limit of quantification

Detection and quantification limit were determined by s (standard deviation of reading of lowest concentration range) and s (the slope of the calibration curve).

Detection limit = 3.3 s/s

Quantification limit= 10 s/s

Precision

Precision was studied by carrying out the analysis of berberine at six concentration RP-HPLC of berberine were analyzed three times on the same day for intraday and three days over a period of one week for interday precision. For the repeatability study, the solution was analyzed for six times and % RSD was calculated.

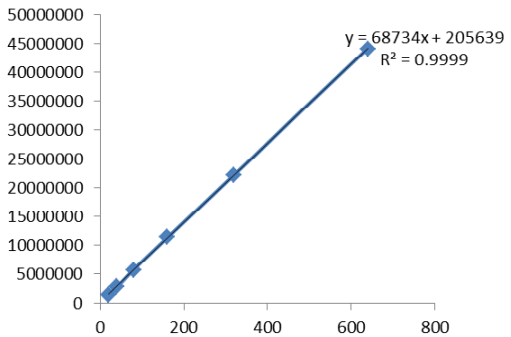


Fig. 1: Linearity graph between concentrations vs. peak area of Berberine

System suitability studies

System suitability parameters like number of theoretical plates (N), resolution (Rs) and tailing factor were studied.

RESULTS AND DISCUSSIONS

The current study revealed the presence of amount of berberine in *T. cardifolia* formulation. In this**,** the separation was carried out on C18 column and mobile phase selected was acetonitrile and water (40:60). The mobile phases were pumped into the column at a flow rate of 1ml/min. The detection wavelength used was 266nm and the method was validated. Retention time for berberin was found to be 8.35 min the result are shown in Figure-2.

Quantity of berberine present in 10 mg of ethanol fraction of methanol extract of *T. cordifolia* was found to be 0.270 mg (Table 4 and figure 3).

Calibration curve for berberine was obtained for the range of 20 to 640 mg/ml. for this calibration slope and intercept value was y=68734x2054. The method showed good linearity over the range of 20-640 mg/ml with (R^2) 0.999. Result was shown in the table-3 and Figure 2.

Table 3: Linearity and range

sample	Range (µg/ml)	Goodness -of fit (r2)	Slope	Intercept
Berberine	20-640	0.999	68734	20563

Table 4 : Estimation of berberine in ethanol fraction of methanol extract of *T. cordifolia*

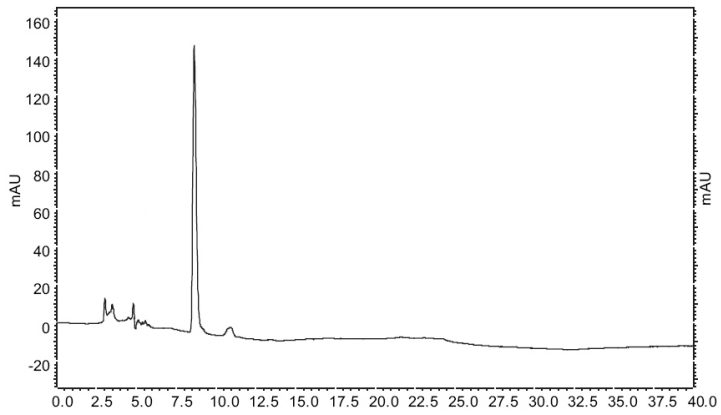
Replicate	Sample area	Berberine (µg)
1	1899012	0.0273
2	1868978	0.0268
3	1889567	0.0272
Average	645947±4329	0.027± 0.01

Mean ± SD, n=3

In present study the limit of detection was 0.8 and limit of quantification was found 1.7. Percentage Recovery studies of berberine found to be 99.38% intraday and interday precision were investigatedby analyzing a target concentration in six replicate preparation of formulated T. cardifolia tablet shown table-5.

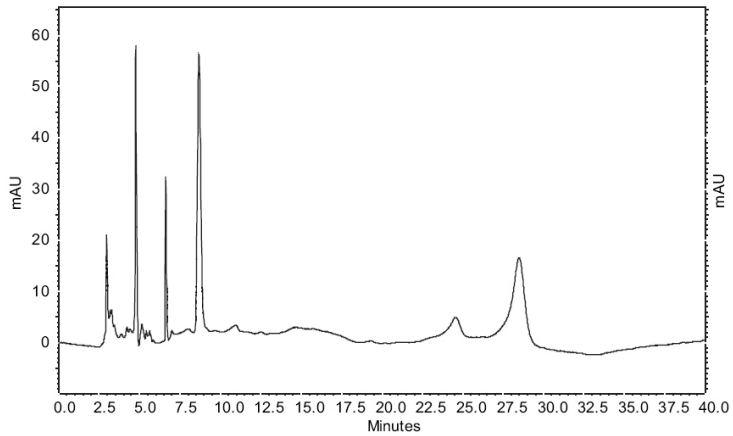
The amount of berberine present in the ethanol fraction of methanol extract of *T. cordifolia* stem in 10 ml was found to be 0.027 µg Table 3. Accordingly 100 mg of ethanol fraction would contain 270 µg of berberine. The HPLC chromatogram of standard berberine and ethanol fraction of methanol extract are shown Figure 2 and fig.3.

Chromatographic data showed that the retention time for tablet was 8.35 at 266 nm. Quantity of berberine in 325mg of tablet was found to be 282.3 ppm as. shown in figure 4.



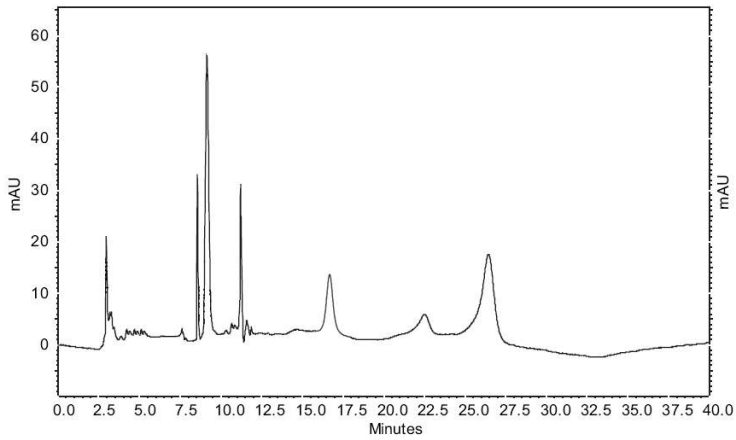
Peak	Retention time	Area	Height	% Area
1	8.35	1487012	629676	100

Fig. 2: HPLC chromatogram of standard Berberine



Peak	Retention time	Area	Height	% Area
1	8.34	1899012	269463	29.174%

Fig. 3: HPLC chromatogram of ethanol fraction of methanol extract of *T. cordifolia*



Peak	RT	Area	Height
1	8.35	1899012	629463

Fig. 4 : HPLC chromatogram of Tinospora Tab

Table 5: Summary of validation parameters

Pricision	Result	Acceptance NMT 2%
Intraday(N=5)	0.15	RSD
Inter day(N=5)	0.12	
Repeatability(n=6)	0.43	
Accuracy(50%-150%)µg/ml	99.3-101	98-102%
Detection limit	0.8	NMT 2% RSD
Quantitation limit	1.7NMT	NMT 2% RSD
System Suitability		NMT 2% RSD
Retention time	8.3	
Theoretical plate	1238	
Tailing factors	1.50	
Specificity	Specific	

Accurate within desired range

Results of validation are summarized in table 5. An analytical method is said to be specific as it can detect the analyte in the presence of expected impurities or additives in test sample.

CONCLUSION

In this study a simple method was developed for estimation of berberine in ethanol fraction of methanol extract of *T.cordifolia* and in its Tablet form. Validation was done as per ICH guidelines. Result showed that the developed method is simple, fast, sensitive, suitable and specific for *T. cardifolia* 325 mg tablet.

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