WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 5, 793-802. Research Article ISSN 2277–7105

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD OF BERBERINE AND ITS QUANTIFICATION IN MULTICOMPONENT HOMEOPATHIC FORMULATIONS

Anjani Chaudhari and Rajashree Mashru*

Faculty of Pharmacy, G.H. Patel Building, Donor's Plaza, The M.S. University of Baroda, Vadodara-390 002, India.

Article Received on
27 Feb. 2017,

Revised on 19 March 2017,
Accepted on 08 April 2017

DOI: 10.20959/wjpr20175-8337

***Corresponding Author'**
Rajashree Mashru
Faculty of Pharmacy,
G.H. Patel Building,
Donor's Plaza, The M.S.
University of Baroda,
Vadodara-390 002, India.

ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the estimation of Berberine in Multicomponent Homeopathic formulations. The separation was achieved by C18 (10 X 4.6 mm, 5 µm) column and 0.1% trifluroacetic acid in water and Acetonitrile (45:55) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 344 nm. Retention time of Berberine is found to be 5.1 min. The method has been validated for Calibration Curve, Accuracy, Precision, LOD and LOQ. Linearity for the method was linear over the concentration range for 0.01-0.06 µg/ml. The percentage recoveries for Berberine is found to be in range of 98-103%. Developed method was found to be Accurate, Precise, Selective and Rapid for Estimation of Berberine in Multicomponent Homeopathic formulations.

KEYWORDS: RP-HPLC, Validation, Multicomponent Formulation.

INTRODUCTION

Berberine is a quaternary ammonium salt from the protoberberine group of benzyl isoquinoline alkaloids. Due to Berberine's strong yellow color, *Berberis* species were used to dye wool, leather, and wood. Wool is still dyed with today in northern India. Under ultraviolet light, shows a strong yellow fluorescence.[1] is a chemical found in several plants including European barberry, goldenseal, goldthread, Oregon grape, phellodendron, and tree turmeric.[2,3] Berberine-containing plants are used medicinally in many traditional medical systems, including Ayurvedic herbal and Chinese herbal medicine. has been used in diabetes,

prostate cancer, cardiac arrhythmia and leukemia.[4] Homeopathic remedies are prescribed on the basis that in a tiny dilution like cures like, so while the very dilute homeopathic remedy may help, the raw product is often best avoided.[5] Analytical methods are reported in literature for the Simultaneous and single molecule particular plant extract[6,7,8] and for whole plant and also studies on plasma has been carried out.[9] we are the first to estimate in multicomponent homeopathic formulation. This work is aimed to develop and validate HPLC method for the determination of Berberine(figure 1) in Homeopathic multicomponent formulation. The method developed is accurate, precise and simple for these compounds in mixtures.

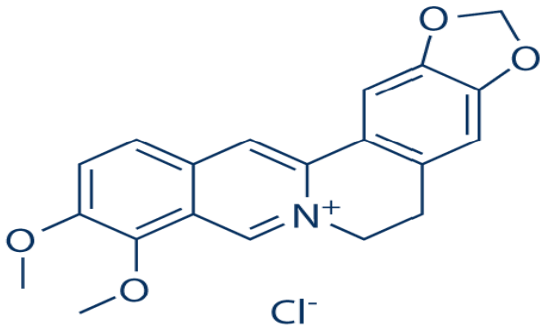

Figure 1: Chemical Structure of Berberine.[10]

EXPERIMENTAL

Chemicals and reagents

Berberine was procured from sigma aldrich with purity of 99.87%. Methanol and Acetonitrile of HPLC grade are of Rankem (mumbai). triflouroacetic acid (HPLC Grade) were procured from Finar Chemicals Ltd (mumbai). Commercial homeopathic formulation was purchased from the local market which contain Berberis aristata and Berberis vulgaris. All other Chemicals and solvents were of analytical reagents grade. All solutions were prepared with HPLC grade water. All solutions were filtered through a Nylon 6,6 membrane filter (Pall Life Sciences, USA) prior to use.

Instrument

A high - performance liquid chromatograph (Shimadzu,Kyoto,Japan) was installed of a LC-20AT prominence solvent delivery system, injector which was manually operated was fixed 20-µl loop and a SPD-20A Prominanace UV-visible detector .Separation was Optimized on a Thermofischer C18 Column (particle Size 5µm: 250 mm × 4.6 mm internal daimeter, Torrance USA), at an ambient temperature chromatogram data were recorded and observed

using a Spinchrom chromatographic Station® CFR Version 2.4.195 (Spinchrom Pvt. Ltd., Chennai, India).

Selection of detection wavelength

Solution of Berberine in acetonitrile was scanned over the range of 200-400 nm. It was observed that the it show considerable absorbance at 344 nm and was selected as the wavelength for detection.(fig.2).

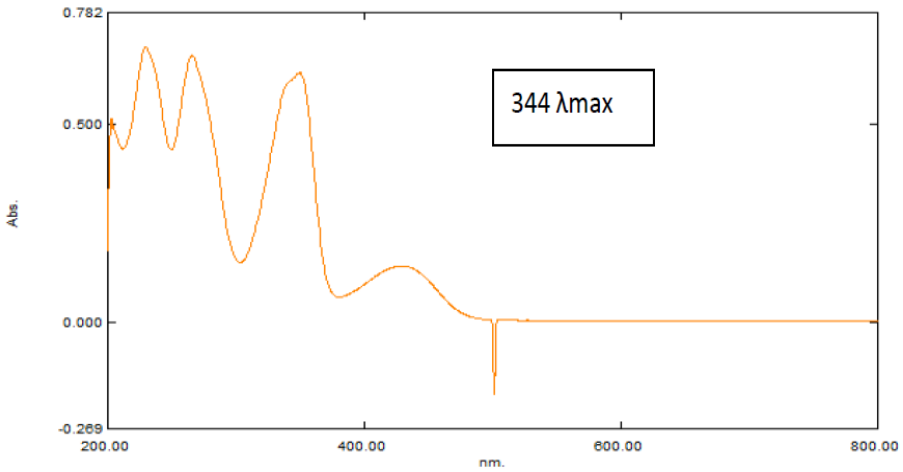

Figure 2 UV spectrogram of Berberine.

Chromatographic Conditions

The isocratic method was developed at 1.0 ml/min flow rate with 0.1% TFA :ACN(45:55,v/v) as mobile phase. The mobile phase was filtered through a 0.2 µm Nylon membrane filter to remove any particulate matter and degassed by sonication for 10 minutes. The absorbance was taken at 344 nm, scanning was done previously on UV to select optimal absorbance wavelength. The sensitivity of detector was set at 0.01 AUFS. Peak area of was the method of Quantification. Before injecting solutions, the column was maintained at equilibrated for at least 30 min with the mobile phase flowing through the system .All the readings was taken in triplicate and the relative standard deviation (%R.S.D) was required to remain below 2.0% on peak area readings.

Preparation of solutions

A stock solutions of Berberine (1000 µg/ml) were prepared in Acetonitrile and were stored at 2-8 OC until used. Aliquots of these solutions were diluted stepwise with mobile phase to obtain 0.01 ppm to 0.06 ppm.

Optimization of chromatographic condition

Sometimes, the effects of different chromatographic conditions on the instrumental responses create a situation where one has to compromise between different experimental variables in order to achieve the best chromatographic separation. Chromatographic separations are significantly affected by the mobile phase conditions, such as mobile phase ratio, the organic modifiers And therefore before selecting the conditions for the optimization, a number of preliminary trails were conducted with different combinations of different organic solvents and buffers at various pH, compositions, and flow rate to check the retention time, shape, resolution. From those experiments the mobile phase combination of 0.1% TFA in water and ACN in the acidic pH range was found to be most suitable.

In order to achieve an optimum separation, following trails were conducted:(table-1).

Table 1: different mobile phase trial.

Mobile phase	Observation
Methanol : Water(50:50)	No peak
ACN:Water (50:50)	No peak
Ammonium Formate pH-2.5 : ACN(50:50)	Distorted peak
Ammonium Acetate pH-4 : ACN(50:50)	Less column
K2HPO4 pH-6 : ACN(50:50)	Fronting
Water (0.1 TFA) : ACN(45:55)	Good shape peak

RESULT AND DICUSSION

Calibration curve (Linearity)

The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations (quantities) of an analyte in the sample. The calibration plot(concentration VS Area) was generated by replicate analysis (n = 6) at all concentration levels . Calibration curve data are shown in table 2.Overlain chromatogram of Berberine(0.01-0.06µg/ml) shown in figure 3 and calibration curve of Berberine represented in figure 4.

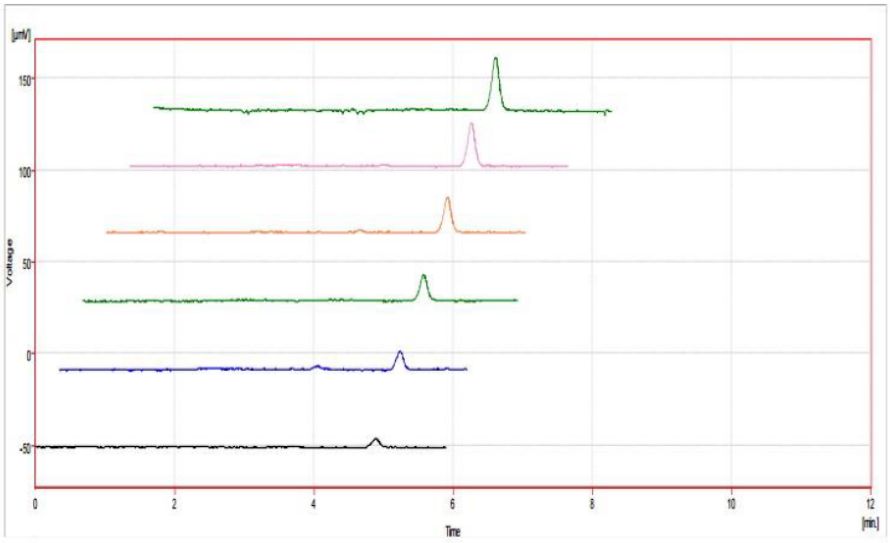

Figure 2: Overlain chromatogram of BERB.(0.01-0.06)(µg/ml)

Table 2: Linearity range of berberine.

conc.(µg/ml)	Area(mV.s)
0.01	0.387
0.02	0.718
0.03	1.063
0.04	1.449
0.05	1.690
0.06	2.123

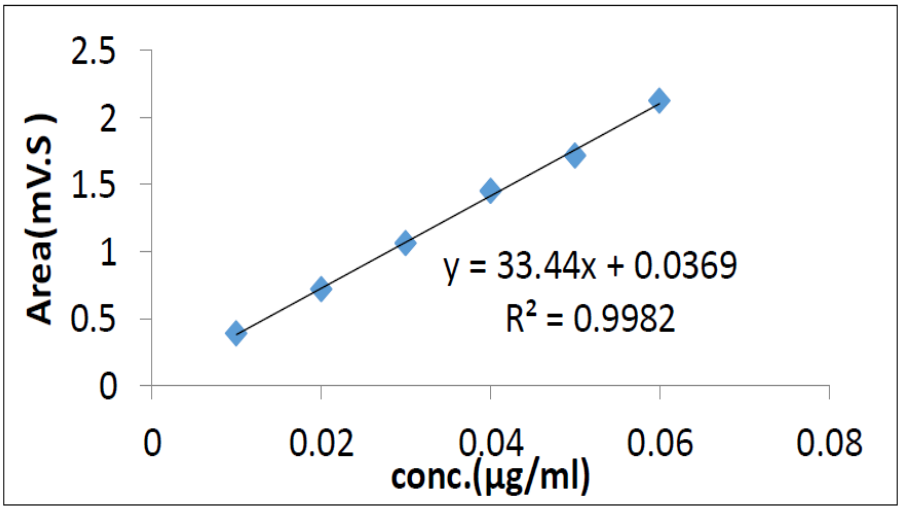

Figure 4. Standard calibration curve of Berberine.

Accuracy and precision

Both repeatability (within a day precision) and reproducibility (between days precision) were determined as follows. Solutions containing lowest, intermediate and highest concentrations

of the calibration curve, i.e. 0.01, 0.03 and 0.06 µg/ml were prepared. Six injections at each of the specified concentration levels were injected within the same day for repeatability and over a period of 3 days (6 injections/day) for reproducibility. Mean and relative standard deviation were calculated and used to judge accuracy and precision of the method. Both intra-day and inter-day samples were compared with standard curves concurrently prepared on the day of analysis. Accuracy can be measured by many methods here we have done percentage of recovery by spiking at 50,100,150 % levels. Results of accuracy and precision are shown in table 3 and 4 respectively.

Table 3: Statistical validation of recovery studies.

Method	% spiking	Actual conc. Of BERB. (µg/ml)	Amount of BERB. (µg/ml)	Amount of recovered (µg/ml)	% Recovery ± SD
RP-HPLC	50%	0.010	0.005	0.005149	102.99±0.3366
	100%	0.010	0.010	0.010658	96.788±0.5202
	150%	0.010	0.015	0.0160	106.82±0.7563

*** Data obtained from 3 replicate injections**

Table 4: Statistical validation of precision data.

Intraday.

Conc. (µg/ml)	Set-1 (Mean ± sd)	Set-2 (Mean ± sd)	Set-3 (Mean ± sd)
0.01	0.3258±0.003	0.3596±0.026	0.280±0.1125
0.03	1.069±0.0257	1.176±0.1190	1. 0718±0.024
0.06	2.1488±0.058	2.0956±0.067	2.187±0.0874

Inter-day

Conc.(µg/ml)	Day 1(Mean ± sd)	Day 2 (Mean ± sd)
0.01	0.3654±0.02972	0.3156±0.0083
0.03	0.9882±0.00755	1.1698±.08684
0.06	2.1432±0.04193	2.0968±0.05997

*** Data obtained from 3 replicate injections**

Detection Limit

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (LOD) may be expressed as:

$LOD = 3.3(\sigma/S)$

Where σ = Relative standard deviation of the response. S = the slope of the calibration curve (of the analyte).

Quantitation Limit

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation Limit (LOQ) may be expressed as.

LOQ = 10(σ/ S)

Where σ = Relative standard deviation of the response. S = the slope of the calibration curve (of the analyte).Data were summarised in Table 8.

Stock solution stability

Stock solution of (100 µg/ml) was prepared in Acetonitrile and stored at room temperature. Area of solution was taken for 1 µg/ml at 0hr, 3hr, 6hr, 12 hr and 24 hr. No change in area was found to occur after 24 hour. Hence, Solution is found to be stable. results shown in the Table 5.

Table 5: data of stock solution stability.

Time interval (hrs)	Area (mV S)
0	33.33
3	33.43
6	33.53
12	33.55
24	33.49
Mean ±sd	33.46±0.088

Specificity

The method was determined as specific by comparing test results obtained from analyses of sample solution containing placebo ingredients with that of test results those obtained from standard solution.

Ruggedness

Ruggedness should be used as a parameter evaluating constancy of the results when external factors such as analyst, laboratory, instrument, reagents and days are varied. We have did the variation of analyst and data shown in Table 6

Table 6: Ruggedness.

Conc.(µg/ml)	ANALYST-1 Peak Area (mV.s) (HPLC)	ANALYSIS--2 Peak Area (mV.s) (HPLC)
0.06	2.30	2.10
0.06	2.27	2.17
0.06	2.29	2.16
0.06	2.28	2.21
0.06	2.23	2.21
0.06	2.33	2.28
Mean ±SD	2.28 ±0.03	2.19±0.06
%RSD	1.45	1.98

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. The parameters included flow rate, mobile phase composition and wavelength. The results are shown in Table 7.

Table 7: Statistical validation of robustness studies.

Factor	Retention time (min)	Peak Area (mV.s)
A. Flow Rate		
0.9	5.51	2.10
1	5.17	2.15
1.1	4.86	2.08
Mean ±SD	5.18 ± 0.32	2.11 ± 0.03
B. MOBILE PHASE COMP. (0.1%TFA IN WATER: ACN)		
50-50	4.51	1.81
45-55	5.17	1.98
40-60	5.52	2.04
Mean ± SD	5.06 ± 0.51	1.94 ± 0.11
C. Wavelength		
342 nm	5.10	2.14
344 nm	5.17	2.07
346 nm	5.17	2.11
Mean ± SD	5.14 ± 0.040	2.10 ± .0.03

*** Data obtained from 3 replicate injection.**

Table 8: Optimized parameters.

PARAMETER	RESULTS
Detection wavelength(nm)	344
Retention time (min)	5.17
Linearity range (µg/ml)	0.01-0.06
Regression equation	Y=33.44x + 0.0369
Correlation coefficient	0.9982
Intraday precision (%RSD)	0.49 %
Inter day precision (%RSD)	0.58 %
LOD (µg/ml)	0.002
LOQ (µg/ml)	0.010
Accuracy (% Mean Recovery)	98- 103 %

System Suitability Test

SST is commonly used to verify retention time, column efficiency and Tailing factor of a chromatographic system to ensure its adequacy for a particular analysis. According to the United States Pharmacopoeia (USP) and the International Conference on Harmonization (ICH), SST is an integral part of many analytical procedures. SST results shown in Table 9.

Table 9: system suitability test (SST) parameters.

PARAMETER	DATA OBTAINED BERBERINE
Retention Time	5.04
Theoretical plate	9888
Tailing factor	0.996

Analysis of marketed formulation Samples.

Extraction Procedure

The 5 ml of sample has been taken and extracted in 5 ml of Acetonitrile by keeping it on ultrasonic bath for 10 to 15minutes. Filtered it through whatmann filter paper. The final solution was make up with mobile phase. This solution was analysed using the developed method. The results for analysis of marketed formulation are shown in Table 10.

Table 10: Quantification in multicomponent formulation.

Formulations	Concentration of Berberine found (µg/ml)	Standard deviation
Formulation 1 (3 x) with Other constituents	0.010	±0.0017
Formulation 2 (2x) with Other constituents	0.017	±0.0013
Formulation 3 (3x) with Other constituents	0.014	±0.0012

CONCLUSION

From the results obtained, it is obvious that the proposed HPLC method is applicable for the determination of Berberine in Homeopathic formulations. The intra-day and inter-day variability and accuracy results were found in acceptable limit. Simplicity of the method, economical nature and low limit of detection and quantification in marketed formulations with other active constituents makes the method superior from other methods. This method analysis Berberine in presence of other active constituents in homeopathic marketed formulations were its concentration were in dilutions as 2X ,3X and not in exact in µg/ml that this method has achieved and its an added advantage.

REFERENCES

1. https://www.revolvy.com/main/index.php?s=Berberine.
2. Agarwal SS, Paridhavi M. Herbal drug technology. University Press. Hyderabad, 2007; 1: 1-2.
3. Pharmacognosy and Plant Cultivation, 1st ed. New Delhi, CBS Publishers and Distributers, 2008; 11-16. 3.s
4. Kumar T. Standardization of herbal drugs – a review. Int journal of universal pharmacy and bio sciences, 2013; 2(4): 7-18.
5. http://www.raysahelian.com/berberine.html.
6. American Herbal Pharmcopoeia, Cs. Hc, 2007.
7. Weber, H. A. and Maureen, J. 2001. Extraction and HPLC analysis of alkaloids in goldenseal-application. Consumer Products and Drug Manufacturing/QA/QC, Agilent Technology, 24: 87-95.
8. Kursinszki, L., Sarkozi, A., Kery, A., and Szoke, E. 2006. Improved RP-HPLC Method for analysisof isoquinoline alkaloids in extracts of Chelidonium majus. Chromatographia, 63: 131-135.
9. Tsai, P. L. and Tsai T. H. 2002. Simultaneous determination of berberine in rat blood, liver and bile using microdialysis coupled to high-performance liquid chromatography. Journal of Chromatography A, 961: 125-130.
10. Berberine: Chemistry and Biological Activity I. V. NECHEPURENKO, N. F. SALAKHUTDINOV a nd G. A. TOLSTIKOV Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, Pr. Akademika Lavrentyeva 9, Novosibirsk 630090 (Russia).