INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

UV-Spectrophotometric Method Development and Validation of Berberine Hydrochloride

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ARTICLE INFO

Published: 20 June 2025
Keywords:
Berberine Hydrochloride, Beer's law, Phosphate Buffer pH 7.4, UV-Spectrophotometer, Validation
DOI:
10.5281/zenodo.15704448

ABSTRACT

To develop and optimize UV- Spectrophotometric method for Berberine Hydrochloride API. To validate newly developed UV- Spectrophotometric method using various parameter according to ICH guidelines. Materials and method: UV-Spectrophotometric method was developed using Phosphate Buffer pH 7.4 the developed method was standardized in terms of validation parameter such as simple, sensitive, precise, linear, accurate, robust, reproducible as per ICH Q2 (R1) guidelines for estimation of Berberine Hydrochloride in marketed formulation this newly developed method was successfully applied. Results: Berberine Hydrochloride exhibits λ max at 275nm and in the concentration range 50 to 250 µg/ml beers law was obeyed. The Limit of Detection was found to be 42.83 µg/ml and Limit of quantification was found to be 142.79 µg/ml. Recovery of Berbeshine in Marketed formulation was obtained in range of 102-115%, All the precision and repeatability results were observed within the acceptance range i.e. less than 2%. Assay of Berbeshine was found to be in range of 95-97%. Conclusion: The method was found to be simple accurate and precise reproducible and marketed formulation of Berberine Hydrochloride was estimated.

INTRODUCTION

1.1 Berberine Hydrochloride:1,2,3

Berberine Hydrochloride is an Isoquinoline alkaloid found in a variety of medicinal plants, mainly in the Berberis genus and the family is Berberidaceae [Berberis vulgaris]. It is Bright yellow in colour and is widely used in traditional medicines. Charka Samhita prescribed that the extract of the plant be taken internally for the treatment of haemorrhage, piles, pruritus and

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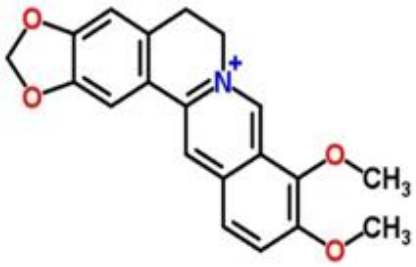
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

alopecia. Sushruta Samhita described it is being useful internally in indigestion deficiency of breast milk and in uterine and vaginal disorders. It also possesses activity against diabetes, High cholesterol, Hypertension, Anti-microbial.

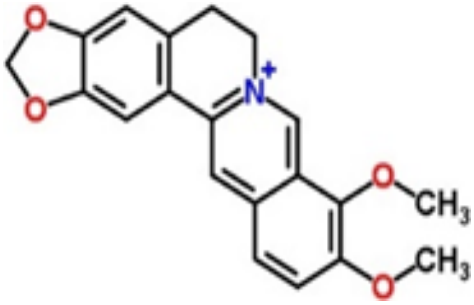
Figure: Berberis vulgaris



Berberine Hydrochloride

1. Drug Profile:[4]

Table no.1 Drug profile of Berberine Hydrochloride

Drug Name	**Berberine Hydrochloride**
Structures:  **2D Str**	 **3D Str**
Iupac Name	5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benz0dioxolo[5,6a]-quinolizinium
Solubility	Phosphate Buffer p^H 7.4
Mechanism Of Action	Berberine Hydrochloride inhibits *pseudomonas aeruginosa* biofilms growth by down-regulating *pslA* and *pelA*. It inhibits inflammation in mice.

Molecular Formula	$C_{20}H_{18}C_1NO_4$	**Molecular Weight**	371.81gm/ml
Category	Diabeties, High cholesterol, Hypertension, Anti microbial	**Colour**	Bright yellow colour
Odour And Taste	Charecteristics odour and taste	**Melting Point**	204-206
State	Solid	**Storage**	Store in dark place
Part Used	Root, Rizhome and Bark		

1.2 UV-Spectroscopy

Basic Principles of spectroscopy[5]

Spectroscopy deals with the production, measurements and interpretation of spectra arising from the interaction of electromagnetic radiation with matter. There are many different spectroscopic methods available for solving a wide range of analytical problems. The methods differ with respect to species to be analysed (e.g. molecular or atomic spectroscopy), the type of radiation matter interaction to be monitored (e.g. absorption, emission or diffraction) and the region of the electromagnetic spectrum used in the analysis. Spectroscopic methods are very informative and widely used for the both quantitative and qualitative analyses. Spectroscopic method based on the absorption or emission of radiation in the ultraviolet (UV), visible(vis), infrared (IR) and radio (nuclear magnetic resonance NMR) frequency ranges are most commonly encountered in traditional food analysis laboratories. Each of these methods is distinct in that it monitors different types of molecular or aromatic transitions.

Spectroscopy Method:[6,7]

It is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples o [tical spectroscopy includes the region on electromagnetic spectrum between 100 A^0 and 400 μm. The region of electromagnetic spectrum.

Fig No.1 UV- Spectrophotometer

Instrumentation of UV- Spectrophotometry[8]

UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measures the ratio, or function of ratio, of the intensity of two beams of light in the UV-Visible region are called Ultraviolet-Visible spectrophotometers. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law. **Beer's law**: It states that the intensity of a beam of parallel

monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration.

Lambert's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. combination of these two laws yields the Beer-Lambert law.

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur.

Mathematically, Beer Lambert law is expressed as

A=a b c

Where,

- A-Absorbance or optical density
- a-absorptivity or extinction coefficient
- b-path length of radiation through sample (cm)
- c- concentration of solute in solution.

Both b and a are constants so a is directly proportional to the concentration c when c in gm/100, then the constant is called A (1%, 1cm)

A=A1%/1cm'bc

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring its absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λ max), where small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9 around which the accuracy and precision of the measurements are optimal. The assay of single component sample, which contains other absorbing substances, is then calculated from the measured absorbance by using one of three principal procedures.

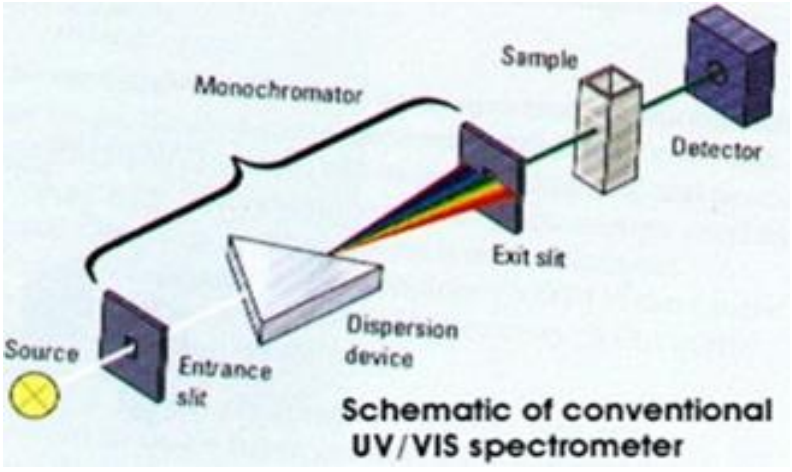


Fig No.2 Schematic instrumentation of UV spectrophotometer

2. METHODOLOGY:

2.1 Materials and methods:

The materials required to carry out the work includes reagents, chemicals and analytical instruments as given below:

Chemicals and Apparatus:

Chemicals: Drug sample Berberine Hydrochloride API was procured from Zyrex Ayurveda India for method development of Berberine Hydrochloride. Methanol, Ethanol, Phosphate Buffer6,8,7,7.4 and Distilled water. **For Method Validation:** Phosphate Buffer 7.4, Berberine Hydrochloride extract, Distilled water, tablet marketed formulation. **Apparatus:** 100ml volumetric flask, 10ml of volumetric flask, beakers, funnels, micro pipette, measuring cylinders, cuvette, soft tissue papers, aluminium foil. **Instruments:** Shimadzu UV 1800 Spectrophotometer.

2.2 Method Development

A. Solubility: In estimation of Berberine Hydrochloride drug it is necessary to know the solubility of the drug. So, the solvent selection was done by performing Gravimetric method in different solvents.

Gravimetric Method[10]: Take 10 ml of each solvent and add 100mg of drug to it. Stir continuously for 24hours.filter the solution and evaporate the solvent using hot air oven at 40^0 c. After drying weigh the residue. Calculate the solubility by using the formula.

Table No.2 Solubility

Solvents	W1	W2	W3=W2-W1
Ethanol	1.08	1.108	0.028
Methanol	1.08	1.103	0.023
P^H7	1.08	1.141	0.061
P^H 7.4	1.08	1.108	0.028
P^H6.8	1.08	1.136	0.056
Distilled water	1.08	1.12	0.043

Formula: W3= W2- W1

By performing above gravimetric analysis we have come to the conclusion that we should use Phosphate Buffer p^H 7.4 should be used.

Calculation:

- W1=weight of empty filter paper.
- W2=weight of filter paper with residue after drying.
- W3=undissolved residue.

Note:

Greater W3 value indicated → More undissolved substances.

Smaller W3 value indicates → Less undissolved substances

B. Methodology for UV-spectroscopy:

First select a suitable solvent then selection of maximum absorbance range and wavelength and determination of wavelength then determination of UV- Spectrophotometric method.

Determination of Maximum Wavelength (λmax):

Take 0.1gm of Berberine Hydrochloride powder API is dissolved in 100ml of Phosphate Buffer p^H 7.4 transfer 1ml of above solution into 10ml of vol flask containing 10ml of Phosphate Buffer p^H 7.4 then the solution was scanned against black for the entire UV visible wavelength 200-400nm. Based on the spectrum λ max of 275nm was selected for further analysis.

C. Standard Preparation:

0.1gm of Berberine Hydrochloride API was weighed accurately then dissolve in **100ml** 0f Phosphate Buffer p^H7.4 transfer **0.5, 1, 1.5, 2, 2.5 ml** of above solution into 10ml vol. flask make up the mark with phosphate Buffer p^H 7.4 to make **50,**

100, 150, 200, 250 μg/ml solutions then take absorbance of these standard measured at λ max of 275nm. The standard curve was drawn by plotting concentration v/s absorbance.

D. Instruments:

Table No:3 Analytical Instruments

Instruments	UV- Spectroscopy
Model	UV1800
Software	UV probe
Company	Shimadzu
Solvent	Phosphate Buffer p^H 7.4
Scanning wavelength range	400-200nm
Maximum absorbance of Berberine Hydrochloride	275nm

E. Method validation: The method was validated according to ICH guidelines (Q2R 1). Method validation was done by using following parameters like Linearity and Range, Precision, LOD, LOQ, Ruggedness, Robustness, Assay, Accuracy.

1. **Linearity and range:** From stock solution transfer **50, 100, 150, 200, 250 μg/ml** of standard solution in 10ml vol. flask make up the volume with phosphate buffer p^H 7.4 up to the mark then scanned at 275nm and calibration curve was plotted by taking concentration X axis and absorbance on Y axis then correlation coefficient was calculated and it was found to be 0.999 calibration was shown in fig No.4 results are in Table No. 4

2. **Precision:** Precision was performed in two ways that is Intraday and interday precision.

Intraday precision: For intraday precision prepare triplicates of 50 **μg/ml, 150 μg/ml, 250 μg/ml** from stock solution in 10ml vol. flask make up the volume with phosphate buffer p^H 7.4 up to the mark then scanned at 275nmand % RSD was found to be less than 2%. The results are shown in **Table No. 5**

Interday Precision: For interday precision prepare triplicates of **50 μg/ml, 150 μg/ml, 250 μg/ml** from stock solution in 10ml vol. flask make up the volume with phosphate buffer p^H 7.4 up to the mark and scanned at 275nm and %RSD was found to be less than 2%. The results are shown in **Table. No 6**

3. **Ruggedness**: The ruggedness was performed change in analyst or change in instrument by preparing the triplicates of upper, middle and lower concentration i.e. 5**0 μg/ml, 150 μg/ml, 250 μg/ml** respectively. The reproducibility was checked which showed %RSD less than 2% and this indicates that the method developed is rugged. The results are shown in **Table No.7**

4. **Robustness:** Robustness is done by changing the wavelength by preparing the triplicates of upper, middle and lower concentration i.e. **50 μg/ml, 150 μg/ml, 250 μg/ml** respectively. The reproducibility was checked which showed %RSD less than 2% and this indicates that the method developed is robust. The results are shown in **Table No. 8**

5. **LOD:** The limit of detection was calculated using equation: **LOD=3.3× σ/S**

Where,

σ – Standard deviation of Y intercept of calibration curve

S - Slope of regression equation, the results are shown **Table No. 9**

6. **LOQ:** The limit of quantification was calculated using equation: **LOQ: 3.3 σ/S**

Where,

σ – Standard deviation of Y intercept of calibration curve

S - Slope of regression equation, the results are shown Table No.10

Assay: 10 tablets of Berbeshine were weighed that contains 500mg of Berberine Hydrochloride. Average weight of tablet is calculated then calculate the theoretical drug content. Prepare stock solution of Berberine Hydrochloride and stock solution of Berbeshine and filter the solution through Whatman filter paper then 100 μg/ml concentration sample solution and standard solution scanned at 275nm then calculate the practical yield.

Formula:

$$\%\text{Purity}=\frac{\textit{Practical drug content}}{\textit{Theoretical drug content}}\times 100$$

Average weight of 10 tablets = 842mg

Lable claim = 500mg Berberine Hydrochloride

(Limit for % purity of drug is 90-120%)

The results are shown in Table No. 10

7. **Accuracy:**

1. **Stock solution of Berberine Hydrochloride:** 0.1gm of Berberine Hydrochloride powder was weighed accurately. Dissolved in 100ml vol. flask containing 100ml Phosphate Buffer p^H 7.4

2. **Sample solution of Berbeshine:** 0.1gm of Berbeshine powder was weighed accurately. Dissolved in 100ml vol. flask in 100ml Phosphate Buffer p^H 7.4.

For 50%: From the above standard stock solution pipette out 0.5ml standard solution and 0.25ml of sample solution transferred into 10 vol. flask and the volume is made up to the mark using phosphate buffer p^H 7.4. solvent three replicates were prepared to performed the study and calculate % recovery.

For 100%: From the above standard stock solution pipette out 0.5ml standard solution and 0.5ml of sample solution transferred into 10 vol. flask and the volume is made up to the mark using phosphate buffer p^H 7.4. solvent three replicates were prepared to performed the study and calculate % recovery.

For 150%: From the above standard stock solution pipette out 0.5ml standard solution and 0.75ml of sample solution transferred into 10 vol. flask and the volume is made up to the mark using phosphate buffer p^H 7.4. solvent three replicates were prepared to performed the study and calculate % recovery.

(Limit for % recovery of drug is 90-120%)

The results were shown in **Table No. 11**

3. RESULTS AND DISCUSSION:

Discussion: The UV- Spectroscopic method was developed for estimation of Berberine Hydrochloride using phosphate buffer p^H 7.4 at 275nm. Validation was performed as per ICH guidelines.

UV-spectroscopic method development and validation: UV method was developed by using Shimadzu UV 1800 spectrophotometer instrument using a solvent system phosphate buffer p^H 7.4 at 275nm.

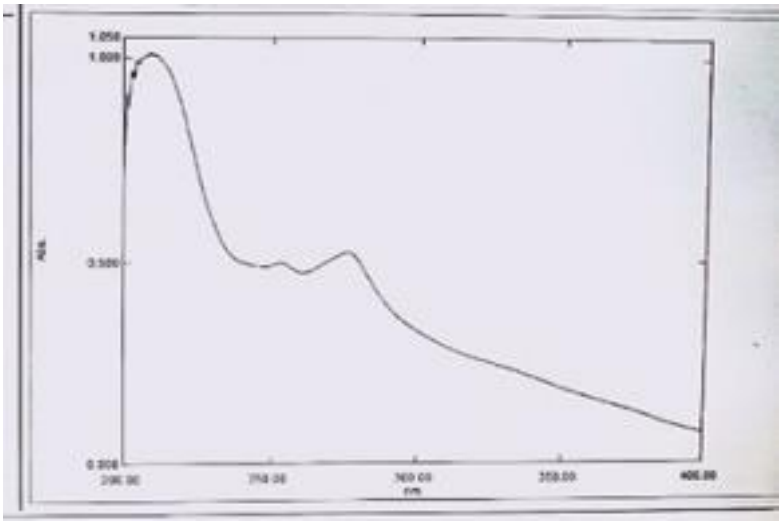


Fig No. 3: UV spectra of Berberine Hydrochloride

Linearity: The linearity range of Berberine Hydrochloride was in the range of 50-250 μg/ml **correlation coefficient :0.999**

Table No. 4

Sr No	Concentration	Absorbance
1	0	0
2	50	0.184
3	100	0.371
4	150	0.522
5	200	0.761
6	250	0.898
	r^2	0.999
	SLOPE	0.0036
	LOD	42.83
	LOQ	142.79

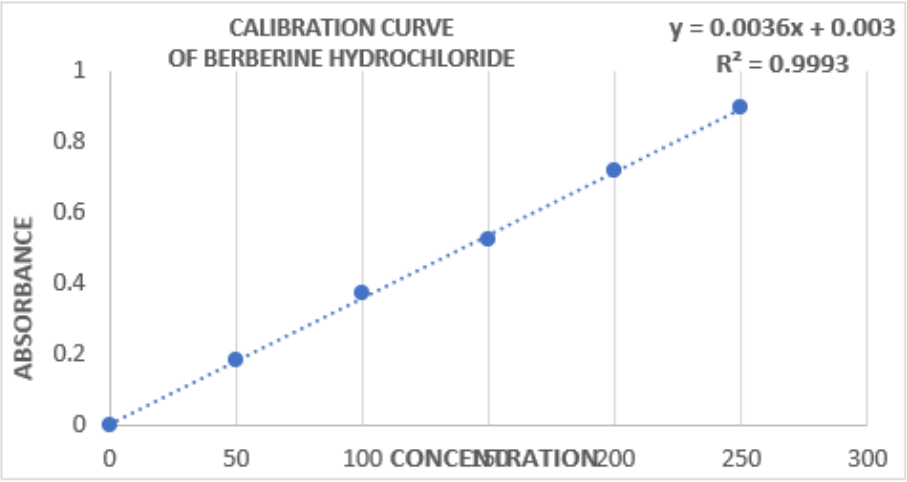


Fig No. 4 Calibration curve of Berberine Hydrochloride

Report: The results of UV spectroscopic method were within the acceptance limit. The calibration curve of the drug gives correlation coefficient value 0.999

Specificity and selectivity: Solvent used in method developed was interfered with the drug hence the method was found to be specific.

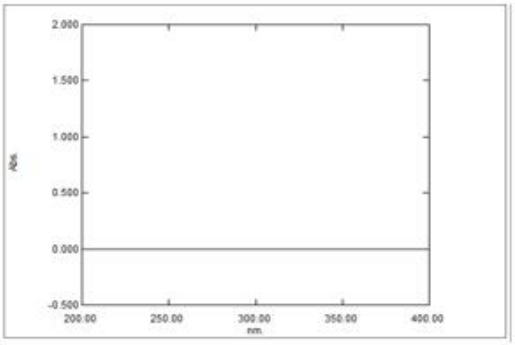


Fig No. 5: Spectra of solvents

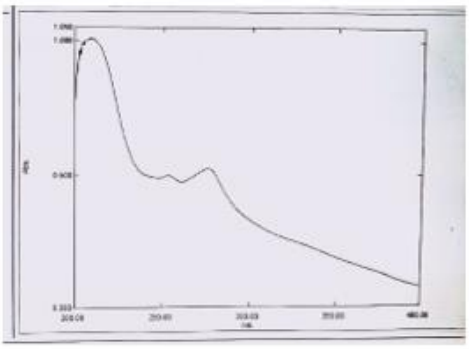


Fig No. 6: UV spectra of Berberine Hydrochloride

Precision: Precision was implemented intraday and interday Precision. The % RSD was found within acceptance criteria

Table No. 5: Intraday precision of Berberine Hydrochloride

Sample no	Conc µg/ml	Absorbance			%RSD	Average %RSD
		Morning	Afternoon	Evening		
1	50	0.252	0.269	0.263	0.75%	0.49%
2	150	0.514	0.521	0.523	0.48%	
3	250	0.749	0.752	0.753	0.26%	

Table No. 6: Interday precision of Berberine Hydrochloride

Sample no	Conc µg/ml	Absorbance			%RSD	Average %RSD
		Day 1	Day 2	Day 3		
1	50	0.253	0.266	0.268	0.8%	0.4%
2	150	0.515	0.518	0.523	0.4%	
3	250	0.750	0.755	0.755	0.2%	

Ruggedness: : By change in analyst ruggedness was performed. %RSD was found within acceptance criteria.

Table No.7: Ruggedness Study

Analysts	Sample no.	Conc µg/ml	Absorbance	SD	%RSD
Analyst 1	1	50	0.189	0.0031	1.65%
Analyst 2	2	150	0.487	0.001	0.21%
Analyst 3	3	250	0.774	0.0015	0.20%

Robustness: : By change in wavelength the robustness was performed. The %RSD was found within acceptance criteria

Table No.8

Sample no	Conc µg/ml	Wavelength			%RSD	Average %RSD
		273nm	275nm	277nm		0.66%
1	50	0.148	0.149	0.147	1.34	
2	150	0.469	0.474	0.472	0.44	
3	250	0.725	0.733	0.731	0.21	

Sensitivity: LOD and LOQ data of Berberine Hydrochloride:

Table No.9

Drug	LOD µg/ml	LOQ µg/ml
Berberine Hydrochloride	42.83	142.79

Accuracy: By taking the absorbance of Standard solution and Marketed solution Accuracy was performed. %Recovery was within acceptance criteria.

Table No. 10 Accuracy of Berberine Hydrochloride

Total conc µg/ml,	Std conc of BH	Sample conc of BH	Absorbance 275nm		Conc Y=mx+c	Sample conc difference µg/ml,	% Recovery
			std	sample			
50%	25	25	0.184	0.186	50.83	25.83	103.3%
150%	25	75	0.371	0.373	102.7	77.7	103.65
250%	25	125	0.522	0.540	149.1	124.1	99.25%

Assay:

Table No:11Assay of Berberine Hydrochloride

Sr no	Sample	Absorbance	%Assay
1	Standard solution	0.512	105.5%
2	Marketed solution	0.229	

CONCLUSION: It was revealed that the newly developed UV-Spectrophotometer method for determining the Berberine Hydrochloride in its pharmaceutical dosage form was simple, specific and cost effective. Using the ICH criteria all validation parameters were found to be acceptable ranges.

Summary: Method was developed by using Phosphate buffer p^H 7.4 as solvent at 275nm.method was validated by using phosphate buffer p^H 7.4 as solvent and accuracy was done by using market formulation (Berbeshine). By using ICH guidelines, the validation was performed

ACKOWLEDGMENT: The completion of our project work has been an intense learning period for us, not only in scientific arena, but also a personal level. In this acknowledgement we take privilege to express sincere gratitude towards the fine individuals for their inspiration guidance and support that we received. First and foremost, we thank the almighty who blessed us with this opportunity. We would like to express our gratitude to our guide Kiran patil Lecturer of Dept

of pharmaceutical chemistry, Rani chennamma college of pharmacy. Her valuable guidance encouragement timely suggestions throughout our work will always remembered. We wish to express our thanks to principal of Rani chennamma college of pharmacy, Belagavi. We express our sincere gratitude to Udaykumar Bolmal sir and all teachers for their valuable Feedback and profound co-operation offered during the time of research work. Thanks to one all……

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HOW TO CITE: Kiran Patil*, Abishek Shedabal, Kalpana Gulappagol, MD Umar Gokak, Pushpa Kadalagi, Vidya Bellivari, UV-Spectrophotometric Method Development and Validation of Berberine Hydrochloride, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 6, 2825-2836. https://doi.org/10.5281/zenodo.15704448