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SIMULTANEOUS ESTIMATION OF BERBERINE HYDROCHLORIDE AND PLUMBAGIN IN MARKETED POLYHERBAL FORMULATION BY RP-HPLC

Deepa R. Patel[1*], Advaita B. Patel[2]

[1]Kalol Institute of Pharmacy, kalol, Pharmaceutical Chemistry department, Gujarat-382721, India.

[2]Kalol Institute of Pharmacy, kalol, Pharmaceutical Chemistry department, Gujarat-382721, India.

**Corresponding Author: Email: deepap.paresh@gmail.com*

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ABSTRACT

A reverse phase high performance liquid chromatographic method has been developed for the estimation of Berberine hydrochloride and Plumbagin in polyherbal formulations. The method is simple, precise and accurate. The method is based on the separation eluted using a mobile phase mixture of methanol and 0.1% ammonium acetate solution in a ratio of 60:40 %v/v at a flow rate of 1.0ml/min. The detection was made at 280 nm. The retention times were 3.01 for Berberine hydrochloride and 8.21min for Plumbagin. Calibration curve was linear over the concentration range of 50-150µg/ml for Berberine hydrochloride and 20-80 µg/ml for Plumbagin. The accuracy of the method was assessed by recovery studies and was found to be for 100.04% for Berberine hydrochloride and 100.06% for Plumbagin. The developed method was validated as per the ICH guidelines parameters like Linearity, precision, accuracy, robustness, LOD and LOQ. The results were validated as per ICH Q2 R1 guideline and were satisfactory.

Keywords *– Berberine hydrochloride, Plumbagin, RP-HPLC, Polyherbal formulation*

1. INTRODUCTION

1.1 Introduction of Berberine hydrochloride

Berberine hydrochloride is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids (Fig. 1). It is found in such plants as Berberis [e.g. *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (Barberry), *Berberis aristata* (Tree turmeric)], *Hydrastis canadensis* (Goldenseal), *Xanthorhiza simplicissima* (Yellowroot), *Phellodendron amurense* (Amur cork tree), *Tinospora cordifolia*, *Argemone mexicana* (Prickly poppy), and *Eschscholzia californica* (Californian poppy). Berberine is usually found in the roots, rhizomes, stems, and bark.[1-4]

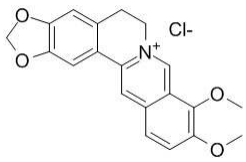

Fig. 1: Structure of Berberine hydrochloride

1.2 Introduction of Plumbagin

Plumbagin or 5-hydroxy-2-methyl-1,4-naphthoquinone is an organic compound. It is regarded as a toxin and it is genotoxic and mutagenic. (Fig. 2) Plumbagin is a yellow dye, formally derived from naphthoquinone. It is named after the plant genus Plumbago, from which it was originally isolated. It is also commonly found in the carnivorous plant genera Drosera and Nepenthes. It is also a component of the black walnut drupe (*Juglans nigra*). Plumbagin is a member of antibiotics group. It is a drug that cures some protozoan infections. Plumbagin is commonly used to treat a variety of infections. Berberine hydrochloride has shown some activity against fungal infections, Berberine hydrochloride seems to exert synergistic effects with fluconazole even in drug-resistant *C. Berberine* hydrochloride is a component of some eye drop formulations. There is some evidence it is useful in the treatment of trachoma. Berberine hydrochloride is a nucleic acid-binding iso-quinoline alkaloid with wide potential therapeutic properties. Berberine hydrochloride and Plumbagin is one of new drug combination. A literature survey revealed that spectroscopic and chromatographic methods are reported for the estimation of these drugs either alone or in combination with other drugs. But no chromatographic method was reported for these drugs to the date. The present paper describes RP-HPLC method for the determination of Berberine hydrochloride and Plumbagin in marketed polyherbal formulation.[5-7]

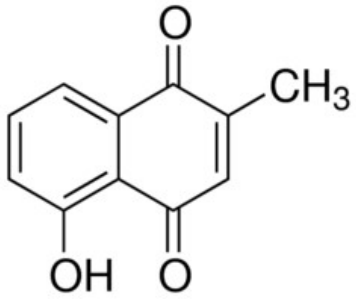

Fig. 2: Structure of Plumbagin

2. MATERIALS AND METHODS

2.1 Apparatus

A gradient high-performance liquid chromatography from Analytical Tech. Limited, equipped with a Diode array detector and Millennium Software was used. A reversed phase Ascentis C_{18} (250 X 4.6 mm i.d, 5 µm particle size) analytical column was used for the present analysis. Digital weighing balance Shimadzu (AX200) and Ultra-sonicator (9 lit) PEI(PECUC300) were used during the study.

2.2 Reagents and materials

Standard Berberine hydrochloride and Standard Plumbagin were obtained from Sigma-Aldrich Pharmaceuticals Pvt. Ltd. Bangalore, India. All solvents were of HPLC grade and all reagents were of analytical grade. Methanol and Ammonium acetate were obtained from Merk (India). Milli Q Water (HPLC Grade) was used throughout the experiment. All solvents and solutions were filtered through a membrane filter (Millipore filter paper type H.V, 0.45µm pore size) and degassed using ultrasonic cleaner before use.

2.3 Optimization of wavelength maxima

Solutions of Berberine hydrochloride and Plumbagin were scanned between 200 and 800nm. UV spectra of both drugs show absorbance at 280nm. (Fig. 3)

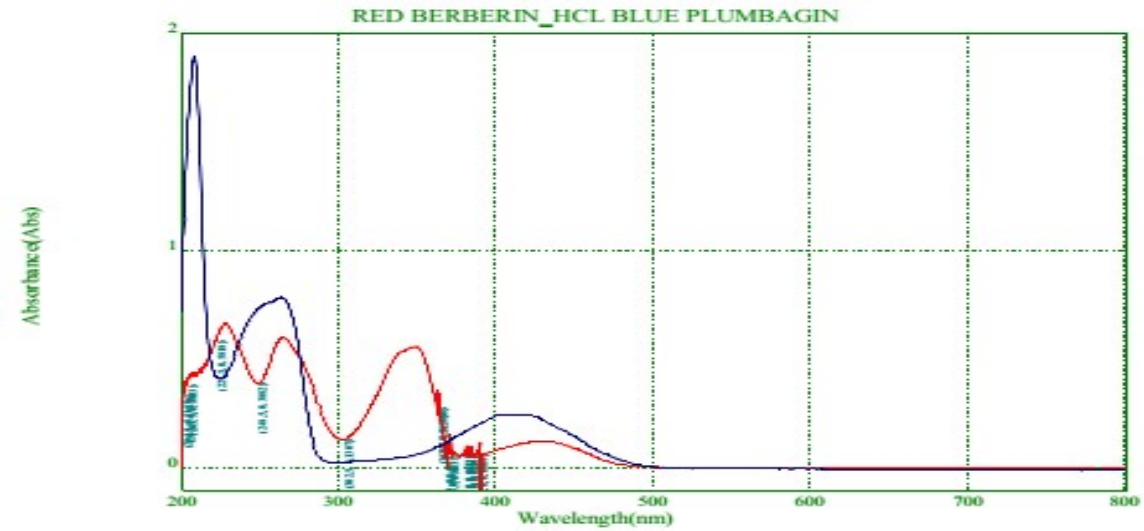

Fig.3: UV overlay spectra of Berberine hydrochloride and Plumbagin

2.4 Chromatographic conditions

The samples were chromatographed on a reversed phase Ascentis C_{18} (250 X 4.6 mm i.d, 5 µm particle size) column with a flow rate of 1.0 ml/min. All analyses were carried out at isocratic conditions. The mobile phase consisted of a mixture of Methanol: 0.1% ammonium acetate solution (60:40% v/v). The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. The volume of injection was 10µl and the detection was made at 280nm.

2.5 Preparation of solutions

2.5.1 Ammonium acetate solution

Take 2.5 gm ammonium acetate dissolved in 30ml water. Volume make with water up to 250ml.

2.5.2 Berberine hydrochloride standard stock solution

Accurately weighed Berberine hydrochloride (25mg) was transferred in 25ml volumetric flask. The drug was dissolved in water with sonication for 5min and final volume was adjusted with mobile phase up to mark to prepare a 1000µg/ml stock solution.

2.5.3 Berberine hydrochloride working standard solution

From the stock solution (1000µg/ml), an accurately measured 0.5, 0.75, 1.0, 1.25, and 1.5ml was transfer into separate 10ml volumetric flask and final volume was adjusted with mobile phase up to mark to prepare 50-150µg/ml solutions.

2.5.4 Plumbagin standard stock solution

Accurately weighed Plumbagin (10mg) was transferred in 100ml volumetric flask. The drug was dissolved in water with sonication for 5min and final volume was adjusted with mobile phase up to mark to prepare a 100µg/ml stock solution.

2.5.5 Plumbagin working standard solution

From the stock solution (100µg/ml), an accurately measured 2.5, 3.75, 5.0, 6.25, and 7.5ml transfer into separate 10ml volumetric flask and final volume was adjusted with mobile phase up to mark to prepare 25-75µg/ml solutions.

2.5.6 Sample preparation

Tablet was taken and weight equivalent to 500mg of powder was transferred into 25 ml volumetric flask. About 20ml of Methanol was added and sonicated for 30 min. Repeat this procedure three times. The solution was cooled to the room temperature and make up to volume with methanol. The solution was filtered through Whatman filter paper (Grade 42); filtrate transferred to 10ml volumetric flask and diluted to 10ml with methanol. Above solution was injected into HPLC system. Peak areas were recorded for all the peaks.

2.6 Optimization of the solvent system

Mobile phase was selected based on the review of literature. Mixture of methanol: water, Acetonitrile (ACN): water, methanol: ACN with various pH, and different volumes at 1mL/min flow rate were tried. The mixture of Methanol: 0.1% Ammonium acetate solution (60:40% v/v) at 1mL/min flow rate, proved to be better than the other mixtures in terms of resolution and peak shape.

2.7 Method Validation [8-10]

2.7.1 Linearity

The linearity was evaluated by linear regression analysis. The calibration curve was obtained with concentrations of pure Berberine hydrochloride and Plumbagin solution ranging from 50-150µg/ml and 25-75µg/ml respectively for the chromatographic method. (Table-1)

2.7.2 Precision

The precision of the procedure was determined by repeatability (intraday). Intraday precision was evaluated by assaying same concentration and during the same day. Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of sample. Another replicate determination on three different days to estimate interday precision.

2.7.3Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a preanalysed sample solution, a definite concentration of standard drug was added and recovery was studied. A 80%, 100% and 120% of pure drug solutions were added to the preanalyzed samples.

2.7.4 Limit of detection and limit of quantification

For HPLC method, the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope by using calibration curves. *2.7.5 Robustness.* Robustness was determined by the analysis of the samples under a variety of conditions making small changes in the buffer pH, in the ratio of mobile phase, and in the flow rate.

2.7.6 System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyzer. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

2.7.7 Analysis of sample solution

The response of sample solution was measured at 280 nm using HPLC. The amount of Berberine hydrochloride and Plumbagin were determined by regression equation.

3. RESULTS AND DISCUSSION

The mobile phase consisting of Methanol: 0.1% Ammonium acetate solution (60:40% v/v) at 1mL/min flow rate which gave sharp, well-resolved peak with minimum tailing factor for Berberine hydrochloride and *Plumbagin*. The retention time for Berberine hydrochloride and Plumbagin were 3.01 & 8.21 min respectively. (Fig. 4) The calibration curve for Berberine hydrochloride & Plumbagin were found to be linear over the range of 50-150µg/ml and 25-75µg/ml respectively. The data of regression analysis of the calibration curves is shown in Table-1. The LOD for Berberine hydrochloride and Plumbagin were found

to be 0.16µg/ml and 0.04µg/ml respectively, while LOQ were 0.5µg/ml and 0.13µg/ml respectively. The results for system suitability test parameters and recovery study are summarized in Table-2 & 3. The summary of validation parameters for analysis of Berberine hydrochloride & Plumbagin were shown in Table-4. The analysis of marketed formulation was shown in Table-5.[11-20]

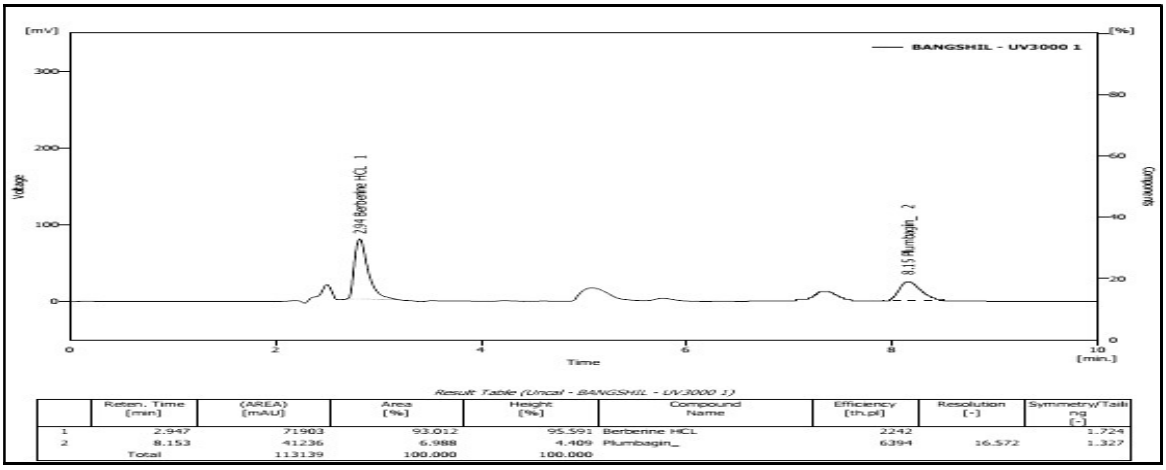

Fig. 4: Chromatogram of Sample solution

Table 1: Linearity data for Berberine hydrochloride and Plumbagin

Drug	Linearity range	Y=mx+c		r2*
		Slope*	Intercept*	
Berberine hydrochloride	50-150µg/ml	778.08	4888.4	0.9992
Plumbagin	20-80µg/ml	1427.6	4664.2	0.9958

***=** Average result of six replicate samples

Table 2: System suitability parameters for Berberine hydrochloride and Plumbagin

Parameters	Data obtained		Standard limits
	Berberine hydrochloride	Plumbagin	
Retention time (min)	3.01	8.22	-
Theoretical plates per meter	6246	12086	>2000
Symmetry/Tailing factor	1.06	0.87	≤2
Resolution	23.23		>2

Table 3: Recovery study for Berberine hydrochloride and Plumbagin

Drug	Conc. Level (%)	Mean Recovery (%)±SD	%RSD
Berberine hydrochloride	80	100.11 ± 0.81	0.81
	100	99.85 ± 0.25	0.25
	120	100.16 ± 0.38	0.38
Plumbagin	80	100.28±0.92	0.92
	100	100.07±0.50	0.50
	120	99.84±0.69	0.69

Table 4: Summary of Validation Parameters

Sr. No.	Parameters	Berberine hydrochloride	Plumbagin
1	Linearity range	50-150µg/mL	20-80µg/mL
2	Correlation coefficient (R^2)	0.9992	0.9958
3	Accuracy (%)	100.04	100.06
4	LOD (µg/ml)	0.16	0.04
5	LOQ (µg/ml)	0.5	0.13
6	Retention time (min)	3.01	8.22
7	Theoretical plates per meter	6246	12086
8	Symmetry/Tailing factor	1.06	0.87
9	Resolution	23.23	
10	% Drug found	0.28	0.12
11	Robustness	Robust	Robust

Table 5: Analysis of marketed formulation

Brand Name	Drug	% Drug found
Bangshil	Berberine hydrochloride	0.28
	Plumbagin	0.12

4. CONCLUSION

The developed method was validated in terms of linearity, accuracy, and precision. The mobile phase is easy to prepare and the drugs are eluted within short run time. A good linear relationship was observed for Berberine hydrochloride and Plumbagin. The percentage RSD for precision is <2 which confirms that method is sufficiently precise. The proposed method is accurate, precise, simple, sensitive, and rapid and can be applied successfully for the estimation of Berberine hydrochloride and Plumbagin in polyherbal marketed formulation without inference.

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