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"SIMULTANEOUS ESTIMATION OF SOME PLANT BASED ANTI-INFLAMMATORY AGENTS PLUMBAGIN AND BARBALOIN BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD"

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ABSTRACT

The present study successfully developed and validated a simple, accurate, and cost-effective UV–Visible spectrophotometric method for the simultaneous estimation of plant-based anti-inflammatory agents, berbaloin and plumbagin. The method was based on their absorption maxima at 291.0 nm and 267.0 nm, respectively, with an isobestic point at 280.0 nm, confirming its suitability for simultaneous analysis. Both drugs obeyed Beer's law within the concentration ranges of 4–12 µg/mL for berbaloin and 10–50 µg/mL for plumbagin, exhibiting excellent linearity with correlation coefficients (R^2) of 0.9985 and 0.9866, respectively. The method demonstrated high precision, as indicated by low %RSD values for repeatability, intraday, and interday studies. Ruggedness and robustness studies further confirmed the reliability of the method, with %RSD values remaining below acceptable limits under varying conditions. The limits of detection (LOD) and quantification (LOQ) indicated good sensitivity of the method for both analytes. Overall, the proposed method is rapid, precise, accurate, sensitive, and robust, making it highly suitable for routine quality control and simultaneous estimation of berbaloin and plumbagin in pharmaceutical and herbal formulations.

KEYWORDS: Berbaloin, Plumbagin, UV–Visible spectrophotometry, Simultaneous estimation, Method validation.

INTRODUCTION

Plant-based compounds have gained significant attention in pharmaceutical research due to their therapeutic potential and minimal side effects. Among these, plumbagin and barbaloin are well-known natural bioactive compounds with prominent anti-inflammatory properties. Plumbagin, a naphthoquinone derivative obtained from plants like Plumbago species, exhibits anti-inflammatory, antimicrobial, and anticancer activities. Barbaloin, an anthraquinone glycoside derived from Aloe vera, is widely used for its anti-inflammatory and healing effects.

The simultaneous estimation of these compounds is important for quality control and standardization of herbal formulations containing them. UV-Visible Spectrophotometry is a simple, rapid, and cost-effective analytical technique commonly used for the quantitative determination of such compounds.

This study aims to develop and validate a UV-visible spectrophotometric method for the simultaneous estimation of plumbagin and barbaloin in plant-based formulations, ensuring accuracy, precision, and reliability in analysis. [1,2]

MATERIALS AND METHODS

Materials

Various analytical grade chemicals and calibrated instruments are used in simultaneous estimation of anti-inflammatory agents i.e, plumbagin and barbaloin. These include Chemicals like Water, DMSO (Rankem), Methanol (Rankem), Ethanol (Bio Liqua Pvt. Ltd.), Chloroform (Rankem). Instruments used are Boiling water bath (Labico), Weighing machine (Sirtech), UV-Visible Spectrophotometer (Shimadzu-1700), melting point apparatus (Amtech), FTIR (Perkin Elmer Spectrum BX), Hot air oven (Scientech), Digital pH meter (lab man), Micropipette (Dragon lab), Ultrasonic bath (Biomall).

Methods

1. Organoleptic Study

A substance's organoleptic features include its taste, odor, color, and appearance. The assessment was carried out using a comparison analysis against predetermined descriptive profiles or accepted criteria. [3]

Visual assessment (Sight), which evaluates color and appearance, and olfactory assessment (Smell), which identifies any distinctive aromas that can point to the existence of volatile organic compounds (VOCs), were the qualities used.

- **Melting point determination of standard drug**

The melting point, which was described as the exact temperature at which a solid transformed into a liquid, was an essential physical property for identifying substances and assessing purity throughout the inquiry. By comparing the recorded melting points with values available in literature, this feature was utilized to identify chemicals. Based on the notion that a pure material had a narrow, sharp melting range, it was also employed to assess purity. Stronger molecular attractions were often indicated by higher melting temperatures, and the reported values also demonstrated the intensity of the intermolecular interactions. [4]

- **Solubility Study**

Solubility is a physical characteristic of many preparations and plays a significant role in science. Solubility is the greatest amount of solute that may dissolve at a given temperature in a known amount of solvent. A homogenous combination of one or more solutes in a solvent is called a solution. Polar solutes often dissolve in polar solvents, whereas non-polar solutes dissolve in non-polar solvents.[5]

- **pH determination**

The stability, solubility, and bioavailability of medications are all significantly impacted by the pH of pharmacological solutions. To assess the posaconazole and aloin solutions' physicochemical properties and make sure they were compatible with biological environments and formulation ingredients, their pH values were set at 50. A pH meter with a glass electrode for the indication electrode and a mercury electrode for the reference electrode was used to take the measurements. To ensure accuracy, each medication solution underwent three tests. By calculating the average pH values and comparing them to standard reference values, the reliability and consistency of the samples were verified.[6]

2. Identification of pure compound via FTIR spectroscopy

The functional groups found in the substances under investigation were described using Fourier Transform Infrared (FTIR) spectroscopy. The method was important because it allowed for quick, non-destructive investigation and the identification of distinctive vibrational frequencies associated with certain chemical interactions. The spectra supported structural elucidation by displaying diagnostic peaks that verified the existence of hydroxyl,

carbonyl, aromatic, and alkene functionalities. The sample was prepared in solid form, and the mid-infrared spectra (4000 – 400 cm^{-1}) was recorded. The device gathered interferograms, which were converted theoretically into spectra showing wavenumber versus absorbance. In order to establish a link with known functional group vibrations, each absorption band was evaluated according to its frequency range, strength, and form. As a result, the FTIR analysis was crucial in confirming the chemicals' identification and purity, supporting the study's other analytical methods. [7]

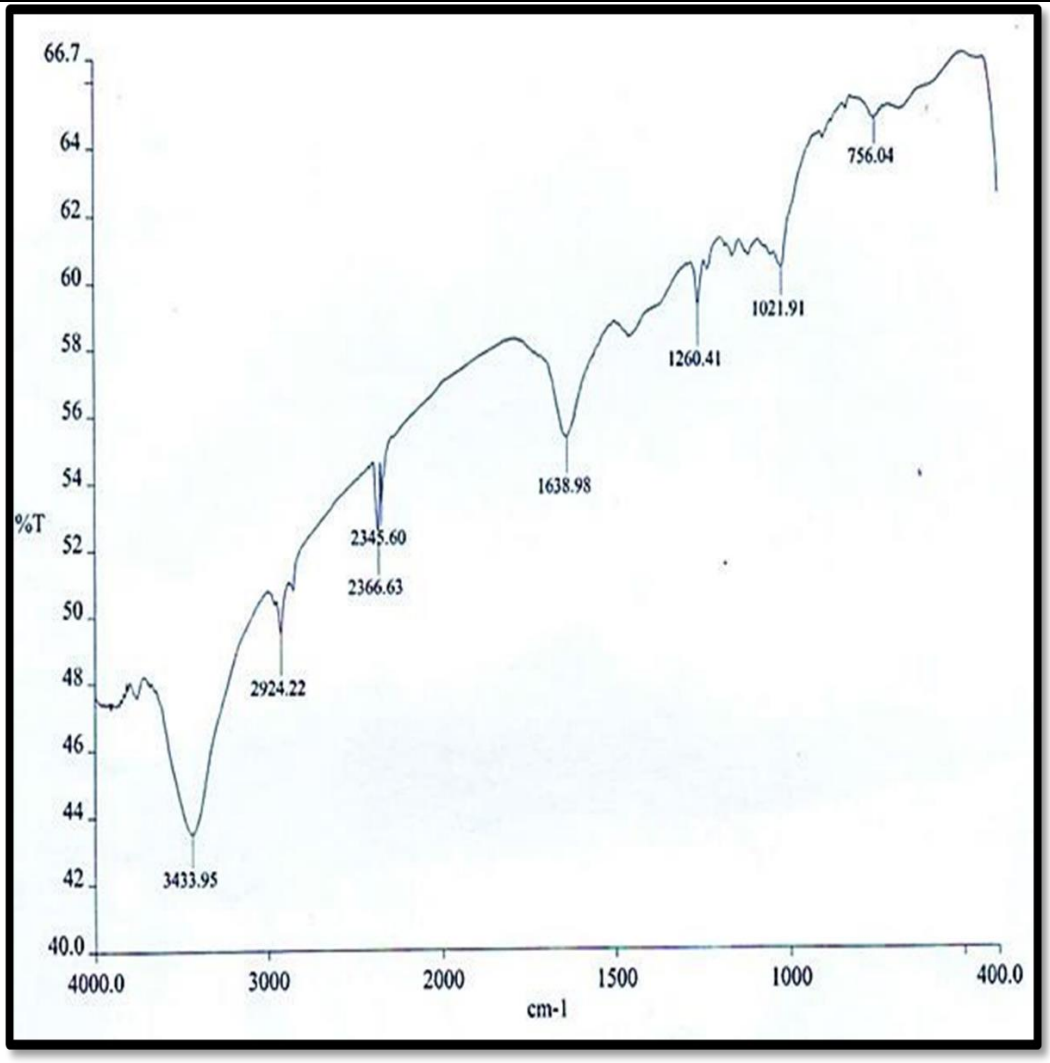


Figure 1: FTIR of Plumbagin.

Table 1: FTIR Interpretation of Plumbagin.

S.NO.	Frequency Range	Group Absorption (cm^{-1})	Group	Compound Class
1	3500- 3400 (cm^{-1})	3433.95	N-H stretching	primary amine
2	3000-2840 (cm^{-1})	2924.22	C-H stretching	alkane
3	1650-1600 (cm^{-1})	1638.98	C=C stretching	conjugated alkene
4	1400-1000 (cm^{-1})	1260.41	O-H bending	carboxylic acid
5	1250-1020 (cm^{-1})	1021.91	C-N stretching	amine

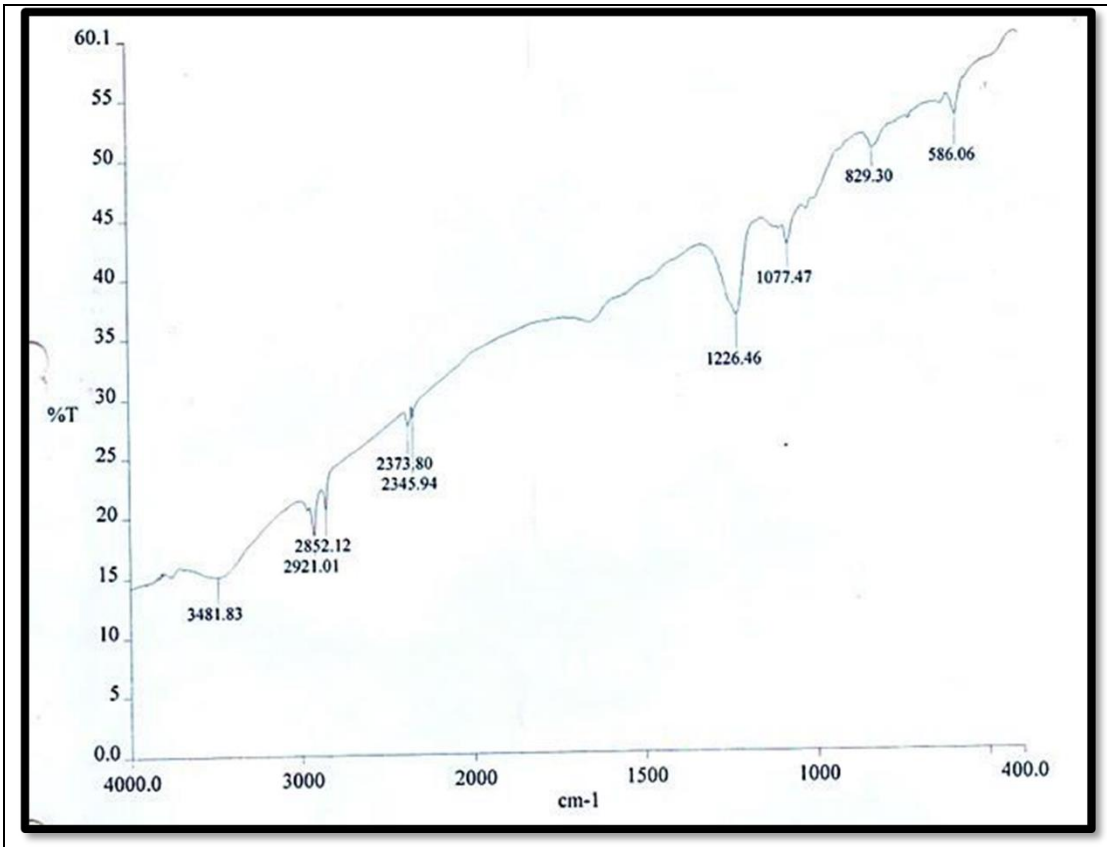


Figure 2: FTIR of Barbaloin

Table 2: FTIR Interpretation of Barbaloin.

S.NO.	Frequency Range	Group Absorption (cm^{-1})	Group	Compound Class
1	3500- 3400 (cm^{-1})	3481.83	N-H stretching	Primary Amine
2	3000-2800 (cm^{-1})	2921.01	N-H stretching	Amine Salt
3	3000-2840 (cm^{-1})	2852.12	C-H stretching	Alkene
4	1275-1200 (cm^{-1})	1226.46	C-O stretching	Alkyl Aryl Ether
5	1085-1050 (cm^{-1})	1077.47	C-O stretching	Primary Alcohol
6	840-790 (cm^{-1})	829.30	C=C bending	Alkene

3. Preparation of standard stock solution

A 10 ml volumetric flask was filled with precisely weighed 10 mg of plumbagin and berbaloin. Acetonitrile was used to modify the volume until the final concentration was

1000µg/ml. This was a typical stock answer. This standard stock solution was used to make further dilutions. [8]

➢ **Method Development by UV spectroscopy**

Berbaloin and Plumbagin were shown to be soluble in acetonitrile. Consequently, the detection wavelength and standard dealing concentration were established using this solvent. According to validation recommendations for analytical procedures published by the International Conference on Harmonization (ICH), this approach is characterized by typical performance that has been confirmed by laboratory study. ICH guidelines were used to validate the created method. [9]

➢ **Determination of wavelength of maximum absorbance (λ max)**

A UV spectrophotometer was used to scan a working standard solution of Berbaloin and Plumbagin (30µg/ml) in acetonitrile separately between 800 and 200 nm.

Table 3: Calibration data of Berbaloin at 291.0nm.

S. No.	Concentration (µg/ml)	Absorbance 1 at 291.0nm	Absorbance 2 at 291.0nm	Absorbance 3 at 291.0nm	Mean Absorbance 291.0 nm
1	10	0.67	0.58	0.71	0.065
2	20	0.101	0.157	0.114	0.124
3	30	0.149	0.178	0.196	0.174
4	40	0.184	0.231	0.281	0.232
5	50	0.345	0.291	0.198	0.278
Mean					**0.292333333**
SD					**0.006576727**
%RSD					**2.226**

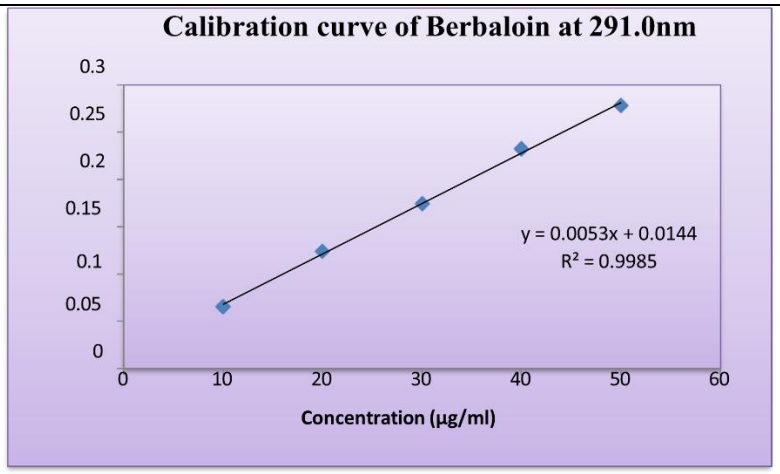


Figure 3: Calibration curve of Berbaloin at 291.0nm

Table 4: Calibration data of Plumbagin at 267.0nm.

S. No.	Concentration (µg/ml)	Absorbance 1 at 291.0nm	Absorbance 2 at 291.0nm	Absorbance 3 at 291.0nm	Mean Absorbance 291.0 nm
1	4	0.071	0.085	0.091	0.082
2	6	0.088	0.095	0.101	0.094
3	8	0.101	0.115	0.121	0.112
4	10	0.127	0.137	0.114	0.126
5	12	0.141	0.149	0.162	0.150
Mean					**0.1132**
SD					**0.006630234**
%RSD					**5.83**

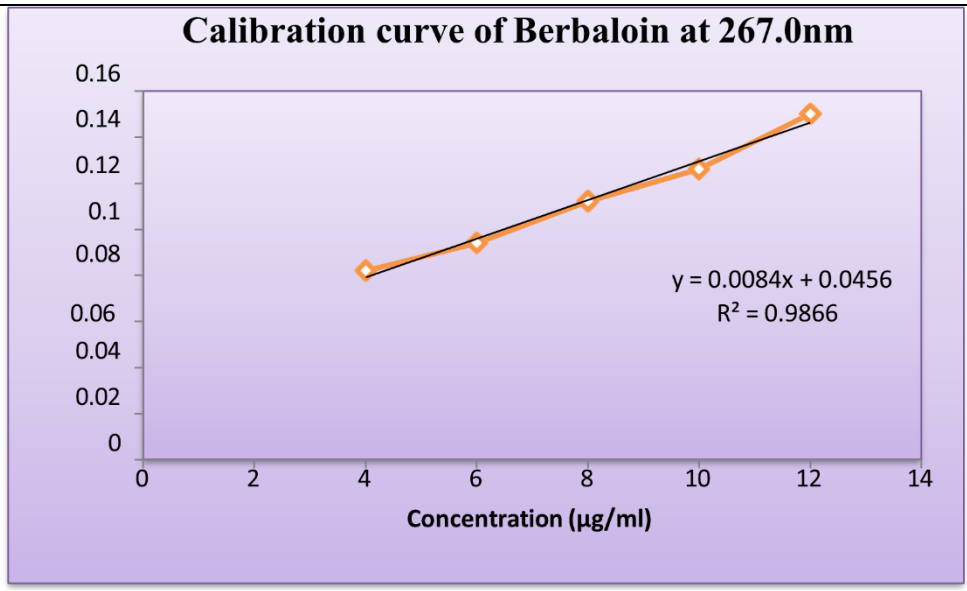


Figure 4: Calibration curve of Plumbagin at 267.0nm

4. Simultaneous equation method

A working standard solution of Berbaloin and Plumbagin (30µg/ml) in acetonitrile was scanned individually between 800 and 200 nm using a UV spectrophotometer.

The details needed are:

a) Berbaloin's absorptivities at λ1 and λ2, ax1 and ax2, respectively

b) Plumbagin's absorptivities at λ1 and λ2, ay1 and ay2, respectively; c) The diluted sample's absorbances at λ1 and λ2, A1 and A2, respectively.

The concentrations of plumbagin and berbaloin in the diluted sample are denoted by Cx and Cy, respectively.

The absorbances at λ1 and λ2 of the mixture are the total of the individual absorbances of Plumbagin and Berbaloin, which leads to the construction of two equations. [11]

$Cx = [(A2*ay1) – (A1*ay2)] / [(ax2* ay1) – (ax1* ay2)]$

Cy = [(A1*ax2 – (A2*ax1)] / [(ax2*ay1) – (ax19*ay2)]

Table 5: Simultaneous estimation of Berbaloin and Plumbagin.

S. No	Berbaloin			Plumbagin		
	Conc. (µg/ml)	Absorbance		Conc. (µg/ml)	Absorbance	
		291.0nm	267.0nm		291.0nm	267.0nm
1	10	0.065	0.08	4	0.060	0.082
2	20	0.124	0.122	6	0.077	0.094
3	30	0.174	0.172	8	0.097	0.112
4	40	0.232	0.227	10	0.110	0.126
5	50	0.278	0.266	12	0.133	0.150
S. No.	**Berbaloin**			**Plumbagin**		
	Conc. (µg/ml)	**Absorptivity**		**Conc. (µg/ml)**	**Absorptivity**	
		291.0nm	**267.0nm**		**291.0nm**	**267.0nm**
1	10	0.0065	0.040	4	0.015	0.020
2	20	0.0062	0.031	6	0.012	0.015
3	30	0.0058	0.028	8	0.012	0.014
4	40	0.0058	0.028	10	0.011	0.012
5	50	0.0055	0.026	12	0.011	0.012

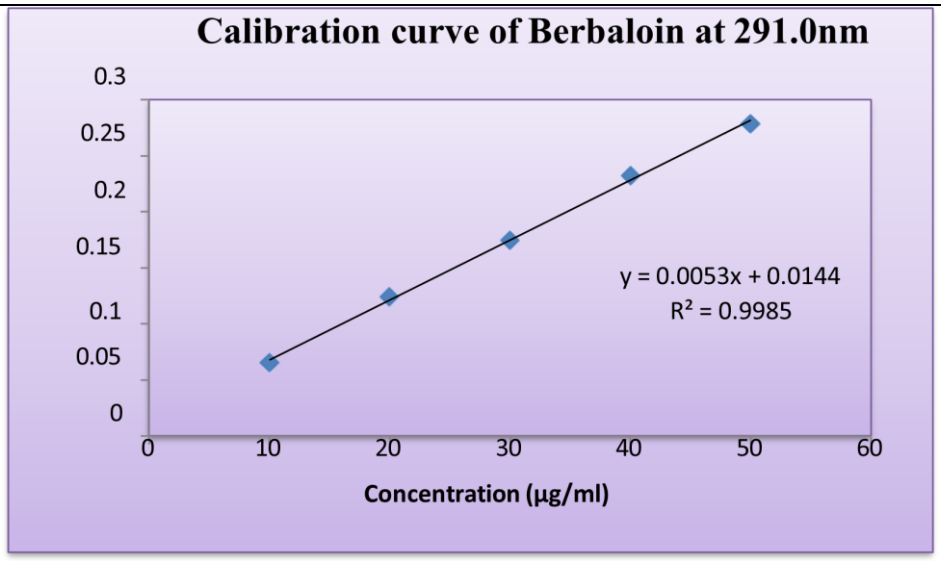


Figure 5: Calibration curve of Berbaloin at 291.0nm

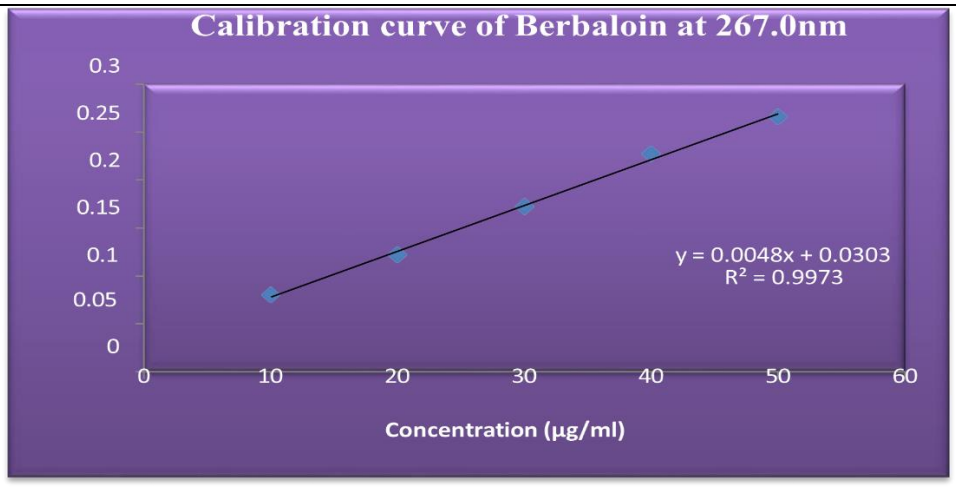


Figure 6: Calibration curve of Berbaloin at 267.0nm

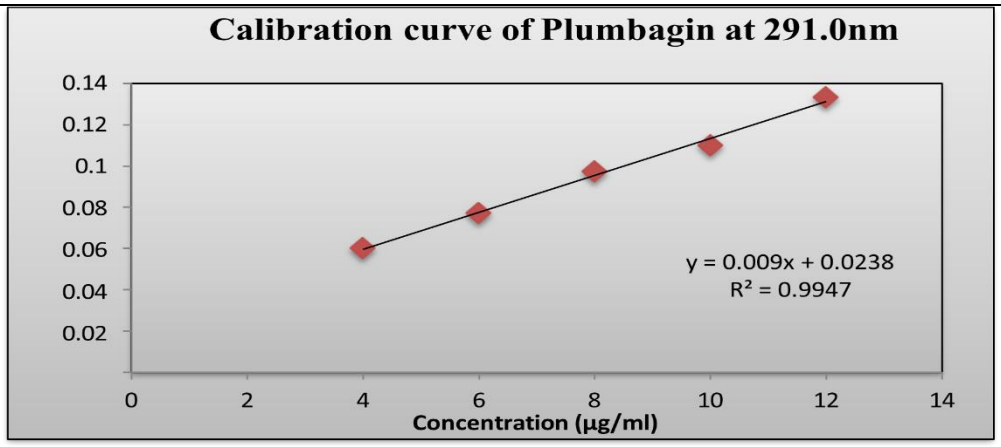


Figure 7: Calibration curve of Plumbagin at 291nm

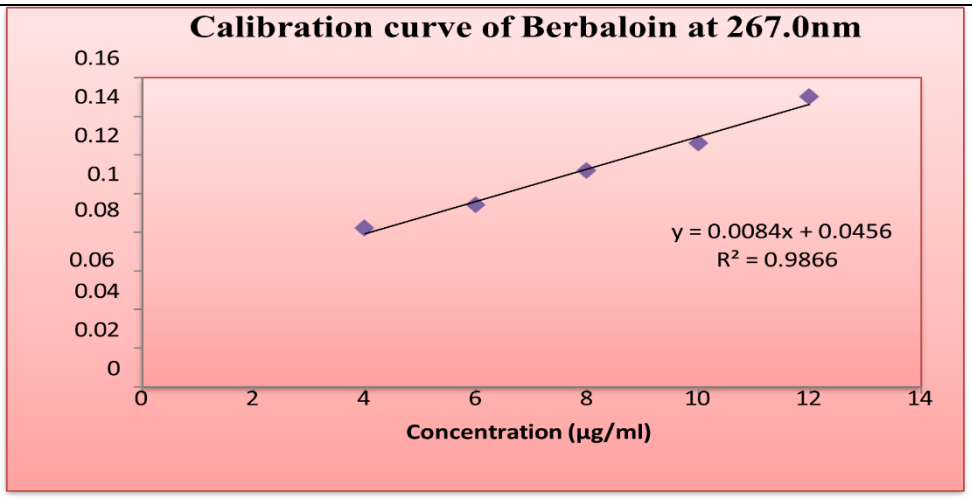


Figure 8: Calibration curve of Plumbagin at 267.0nm

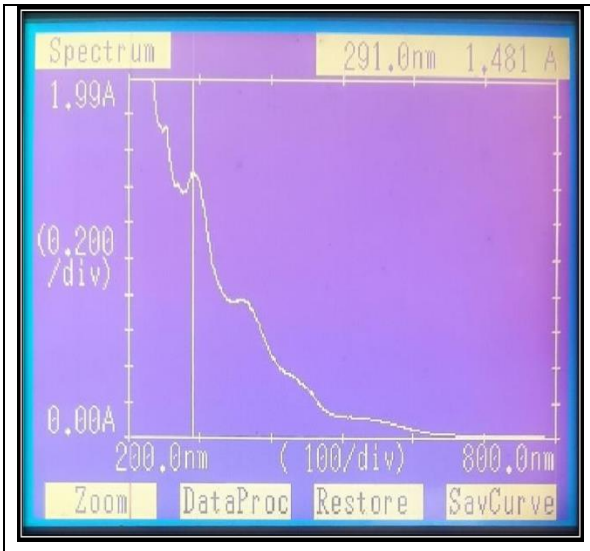
RESULTS AND DISCUSSION

Berbaloin and Plumbagin, two plant-based anti-inflammatory drugs, were effectively estimated together in this work utilizing a UV-visible spectrophotometric approach. Berbaloin and Plumbagin were discovered to have λmax values of 291.0 and 267.0 nm, respectively, with an isobestic point at 280.0 nm (Figure 9). Both medications exhibited strong linearity with correlation coefficients (R^2) of 0.9985 and 0.9866, respectively, and followed Beer's law in the concentration ranges of 4–12 µg/mL for berbaloin and 10–50 µg/mL for plumbagin. With %RSD values for repeatability (0.430% for Berbaloin and 1.036% for Plumbagin), intraday (0.408% and 0.610%), and interday (0.571% and 0.621%), respectively, the technique demonstrated adequate accuracy and dependability. The approach is stable under small experimental changes, as demonstrated by the low %RSD values (<1%) for both analysts and the acceptable variance (<1.2%) found in robustness experiments at 25°C and 45°C. The LOQ values were 2.553 µg/mL and 2.592 µg/mL, respectively, indicating high sensitivity; the LOD values were 7.738 µg/mL for berbaloin and 7.857 µg/mL for plumbagin (Table 6). All things considered, the new UV spectrophotometric approach is easy to use, quick, accurate, precise, and economical. It may be used to simultaneously estimate plumbagin and berbaloin in pharmaceutical and herbal formulations.

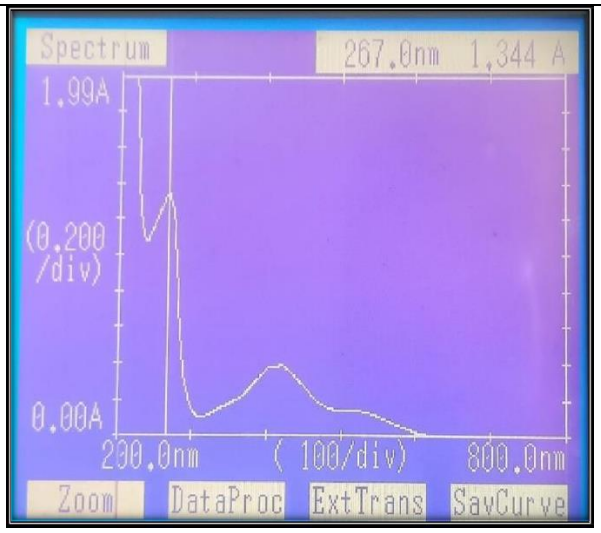
Table 6: Optical Characteristics and Validation Study of Drugs.

S.No	Parameters	Berbaloin	Plumbagin
1	Wavelength λ max nm	291.0	267.0
2	Beer's law limit µg/ml	4-12	10-50
3	Correlation coefficient (R^2)	0.9985	0.9866
4	Slope	0.0053	0.0084
5	Intercept	0.0144	0.0456
6	SD	0.06576	0.00663
7	% RSD	2.226	5.83
8	Precision Repeatability Intraday (%RSD) Interday (% RSD)	 0.430 0.408 0.571	 1.039 0.610 0.621
9	Ruggedness Analyst 1 (% RSD) Analyst 2 (% RSD)	 0.340 0.335	 0.625 0.609
10	Robustness Temp. 25^0C (% RSD) Temp. 45^0C (% RSD)	 0.900 0.574	 1.111 1.052
11	LOQ (µg/ml)	2.553	2.592
12	LOD (µg/ml)	7.738	7.857

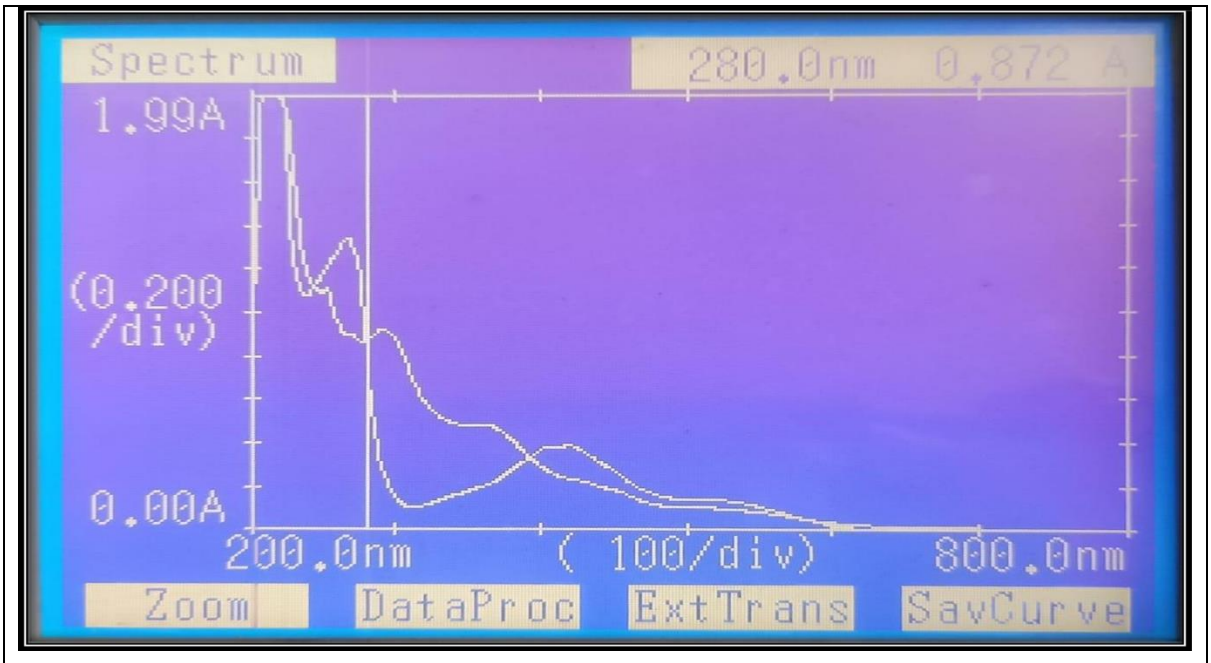
Figure 9: Determination of wavelength of maximum absorbance (λ max)



UV spectrum of Berbaloin (λ max at 291.0nm)



UV spectrum of Plumbagin (λ max at 267.0nm)



UV spectrum of Berbaloin and Plumbagin overlay (Isobestic point at 280.0nm)

CONCLUSION

A UV–visible spectrophotometric technique for the simultaneous measurement of plumbagin and berbaloin was effectively devised and verified in this work. Berbaloin and Plumbagin have λ max values of 291.0 and 267.0 nm, respectively, with an isobestic point at 280.0 nm, indicating appropriate analytical wavelengths. Within the concentration ranges of 4–12

µg/mL and 10–50 µg/mL, respectively, both medications exhibited high linearity and complied with Beer's law (R^2 = 0.9985 for Berbaloin and R^2 = 0.9866 for Plumbagin). The technique demonstrated good accuracy, with %RSD values for repeatability (0.430% and 1.036%), intraday (0.408% and 0.610%), and interday (0.571% and 0.621%) trials being within acceptable bounds. With low %RSD readings under various analyzers and temperature circumstances (25°C and 45°C), robustness and robustness experiments further validated the method's repeatability. With LOD values of 7.738µg/mL and 7.857µg/mL for Berbaloin and Plumbagin, respectively, and LOQ values of 2.553µg/mL and 2.592µg/mL, the technique demonstrated high sensitivity. The suggested approach is appropriate for the regular simultaneous measurement of Berbaloin and Plumbagin in pharmaceutical and herbal formulations since it is straightforward, accurate, exact, affordable, and dependable.

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