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Development and Validation of Uv- Spectrophotometric Method for Estimation of Jatamansone in Nardostachys Jatamansi

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ABSTRACT

Aim: This study aimed to develop and validate a simple, precise, and cost-effective UV- visible spectrophotometric method for the estimation of jatamansone in Nardostachys jatamansi extract. The analysis parameters were selected following the ICH Q2 (R1) guideline.

Methods: Jatamansone solution was scanned across the UV-visible range to identify its wavelength of maximum absorbance. Calibration standards of jatamansone were prepared, and a concentration vs. absorbance calibration curve was plotted. The developed UV-visible spectrophotometric method was utilized to estimate jatamansone in standardized extracts of Nardostachys jatamansi.

Results: The maximum wavelength of jatamansone was determined to be 278nm. The developed UV-visible spectrophotometric method exhibited a correlation coefficient of 0.999 over a concentration range of 1-6 µg/ml. Validation of the method included assessments of linearity, accuracy, precision, robustness, ruggedness, limit of detection, and limit of quantitation. The developed UV-visible method demonstrated good precision for both intra- and inter-day studies, with standard deviations ranging from 0.75 to 1.798 and 0.889 to 1.572, respectively. The content of jatamansone in the Nardostachys jatamansi extract was found to be 4.72% using the proposed UV-visible spectrophotometry method.

Conclusion: A simple, precise, and cost-effective UV-visible spectrophotometric method was successfully developed for the estimation of jatamansone in standardized extracts of Nardostachys jatamansi. The method utilized a solvent with an economical percentage of the organic phase in aqueous media. The validated UV-visible method can be efficiently employed for the estimation of jatamansone in Nardostachys jatamansi extracts.

Keywords - UV- Visible Spectrometric Method, Jatamansone, Nardostachys jatamansi, Validation

I. INTRODUCTION

Nardostachys jatamansi, commonly known as Jatamansi, is a plant primarily found in central and peninsular India, specifically in the dry mixed deciduous tropical forests of Gujarat, Madhya Pradesh, Karnataka, Kerala, Maharashtra, and Sub-Himalayan areas. It belongs to the family Valerianaceae[1-7]. Various parts of Nardostachys jatamansi, including the rhizomes (underground stems), are prominently used for their therapeutic benefits. One of the vital phytochemicals found in the rhizome of Nardostachys jatamansi is jatamansone. Jatamansone is chemically known as 4a,8a-dimethyl-7-(propan-2-yl)-decahydronaphthalen- 1-one.

Fig.1. Jatamansi (Nardostachys jatamansi) plant its rhizome and its phytoconstituent Jatamansone

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The potential uses of jatamansone in the prevention and treatment of a number of medical problems, such as diabetes, high blood pressure, high cholesterol, Alzheimer's disease, and as a supportive supplement during cancer treatment, have drawn attention (18–24). It is only weakly soluble in water and shows solubility in organic solvents such methanol, ethanol, and dimethyl sulfoxide. Tatamansone's pKa values are 7.5 and -7.5, and its partition coefficient is 3.52.

Despite the well-established physicochemical properties and therapeutic importance of jatamansone, there is a scarcity of analytical techniques for its quantification worldwide. Particularly, there is a lack of a specific UV-visible spectrophotometric method for the accurate estimation of jatamansone in Nardostachys jatamansi, specifically in its Ultrasound Assisted (UAE) and Soxhlet Assisted (SAE) extracts.

Considering the future potential of jatamansone, the development and validation of an accurate, precise, and cost-effective UV-visible spectrophotometric method becomes crucial. Such a method would enable the reliable quantification of jatamansone in various extracts of Nardostachys jatamansi. In light of this, the present study aims to bridge this gap by developing and validating a UV-visible spectrophotometric method for the quantitative analysis of jatamansone in different extracts of Nardostachys jatamansi.The proposed method holds significant promise for the accurate determination of jatamansone content,offering a valuable tool for quality control and standardization of Nardostachys jatamansi extracts. Additionally, this method could facilitate further research and exploration of the therapeutic potential of jatamansone in various applications.

II. MATERIALS AND METHOD

Materials:

The supplier of jatamansone was TCI Chemicals (India) Pvt.Ltd, located in Chennai. We bought methanol from Merck. For the purpose of the study, all analytically grade compounds were used.

Instruments Used:

For the analysis, a computer running Spectra Manager software was connected to a double beam UV-visible spectrometer (UV-530, Jasco). For spectral measurement, quartz cells measuring 3 cm in length and 1 cm in path length were employed. For precise weighing, an electronic balance (Essae, Vibra HT) with an internal calibration mode was utilized.

Preparation of standard stock solution:

To generate a stock solution of 1000 µg/ml (Stock-I), also known as Primary Stock, 10 mg of precisely weighed Jatamansone was placed into the calibrated volumetric flask and dissolved using a 10 ml co-solvent system made up of methanol and water (40:60 v/v). To get a solution of 100 µg/ml working stock, the Stock-I solution was appropriately diluted using a co-solvent system.

Determination of wavelength of maximum absorbance (λmax):

Using the co-solvent system as a blank, the full scan mode was used to scan the Stock-II solution over the whole UV and visible spectrum, or 200 to 800 nm. Following spectrum acquisition, λmax was determined using software; the spectrum is displayed in (figure.2). The aforementioned procedure was carried out five times in order to provide repeatable results.

Preparation of calibration curve:

The calibration curve was created by diluting the stock-I solution to meet the six distinct calibration standards, which stood for the following strengths: 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml, and 6µg/ml for CAL STD. Every calibration standard's absorbance was measured using a fixed wavelength measuring mode at the predetermined λmax of 278 nm. Plotting the calibration curve showed concentration vs absorbance. To achieve repeatable results, the aforementioned technique was carried out five times.

Method Validation:

The ICH standard was followed in the validation of the developed UV technique for the measurement of jatamansone. A variety of criteria were assessed, including limit of detection (LOD) and limit of quantitation (LOQ), linearity range, accuracy, precision, robustness, and ruggedness [19–23].

Linearity and Range:

Six distinct calibration standards were used to establish the linearity of the suggested UV technique. Calibration curves in the form of absorbance vs. concentration plots were created based on the study of calibration standards, and they were then subjected to linear least square regression analysis. The suggested method's

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linearity was thought to be established by the R2 value. The range of the suggested UV technique was stated to be the range between the upper and lower concentration limit with satisfactory linearity.

Accuracy:

Recovery studies were used to assess the accuracy of the suggested UV technique following the standard addition of the relevant analyte. Three distinct Jatamansone solutions, each at 80%, 100%, and 120% of the prescribed concentration, were made in triplicate. Using the conventional addition procedure, various concentrations of Jatamansone were added, and the accuracy was determined based on the percentage of recovery. This formula was used to get the recovery percentage.

(SPS-S/SP) × 100 = % RC

Whereas
SPS = The quantity in the tampered sample S = Amount discovered in the specimen
SP = Sum added to the specimen
Percent recovery = % RC

Precision:

UV analysis of predetermined samples was done both within and between days to determine the precision of the suggested UV approach. There were three concentration levels used during the investigation. An intra-day precision research was conducted by preparing nine separate solutions of Jatamansone at concentrations of 1.5µg/ml, 3µg/ml, and 5.5µg/ml (three solutions of each strength) and assessing them in the morning, afternoon, and evening of the same day. Utilizing the percent relative standard deviation, the percent relative standard deviation (%RSD) was computed. Similarly, by looking at the aforementioned answers over the course of three days, an inter-day accuracy analysis was conducted.

Robustness:

The robustness of the UV technique that was developed was tested with varying proportions of methanol in the co-solvent system. Jatamansone was dissolved separately in the same co-solvent system, and methanol concentrations in the co-solvent system were maintained at 41:59 and 39:61 percent, respectively. Three samples' absorbance was measured at 278 nm. Using the previously established calibration curve, the amounts of jatamansone in each sample were determined. The findings were computed using the percentage RSD.

Ruggedness:

A research on the robustness of the approach was conducted by evaluating three duplicate samples of Jatamansone solution (3µg/ml) using two distinct instruments (BA-UV-2600, BIOAGE, and V-530, Jasco), and recording the absorbance in terms of percentage RSD.

Limit of Detection (LOD):

The LOD of the developed UV method was calculated by using following formula LOD=3.3×SD/S

Where, SD= Standard deviation of Y-intercepts S= Slope

Limit of Quantitation (LOQ):

The LOQ of the developed UV method was calculated by using following formula LOQ= 10×SD/S
Where, SD= Standard deviation of Y-intercepts S= Slope

Estimation of Jatamansone in Nardostachys jatamansi extract:

Drying treatment for preparation of Nardostachys jatamansi powder extract

Using a Microtray drier (S.B. Panchal & company, Mumbai, India) and a twin-blade mixer (Bajaj electrical ltd., Mumbai, India), nadostachys jatamansi was dried at 50 degrees Celsius and then powdered. The powder was sieved for 15 minutes using sieves of various sizes (12, 24, 45, 85, and 120 mesh; Swastika Electric and Scientific Works, Ambala, India) in a sieve shaker (CIP Machineries, Ahmedabad, India) in order to choose homogenous particle size. Powder that was collected and used for additional extraction was run through a 120 mesh filter.

1. Soxhlet assisted extraction (SAE) :

Using a Micro tray drier (S.B. Paschal and company, Mumbai, India) and a dual blade mixer (Bajaj electrical ltd, Mumbai, India), nandostachys jatamansi powder was dried at 50oC. To choose a uniform particle size, powder was filtered for 15 minutes using sieves of different diameters (12, 24, 45, 85, and 120 mesh; Swastika Electric and Scientific Works, Ambala, India). The sieve shaker was made by CIP Machineries, Ahmedabad, India. A 120 mesh filter was utilized to capture and use the powder for extraction. The Soxhlet assisted

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extraction (SAE) method was used to carry out the extraction. A 10g thimble of powdered Nardostachys jatamansi powder (Borosil, Mumbai, India) was placed within a Soxhlet apparatus. The substance was fully extracted using methanol. SAE was conducted for two hours. After a predetermined extraction interval, the solvent was distilled off under decreased pressure using a rotary vacuum evaporator (Heidolph instruments GmbH & co., Germany) to obtain the dry extract. 1 was weighed exactly.A 1000 g/ml stock solution (Stock-III) was created by dissolving 1 milligram of Nardostachys jatamansi Powder dry extract in 10 ml of methanol in a calibrated volumetric flask. The suggested UV method was used to determine the Jatamansone content after diluting the stock-III solution with a co-solvent system.

2. Ultrasound Assisted Extraction (UAE) :

The extraction of Nardostachys jatamansi was conducted using a tunable ultrasonic bath (PCi^{Tm}Analytics, 230V AC, 50 Hz, Mumbai, Maharashtra, India). 10 gm of powder was weighed and placed in 100 ml of conical flask. The extraction of Nardostachys jatamansi powder was carried out by placing the beaker in an ultrasonic bath with the fixed power of 150W. The conical flask was immersed in the ultrasonic bath and extracted for 20 min. After extraction process, extract was cooled to room temperature and kept for centrifugation by using micro centrifuge at 25^{0}C using 10,000 rpm for 10 min. Lastly, the supernatant was collected and filtered by using 0.45 µm fitted with syringe filter. The filtrate was suitably diluted with a co-solvent system and analyzed for the Jatamansone content using the proposed UV- visible spectrophotometry method.

III. RESULTS AND DISCUSSION

Determination of wavelength of maximum absorbance:

Quantitative UV analysis requires the identification of the wavelength at which absorption is maximal. To find the wavelength of maximum absorbance, a solution is generally thought to be appropriate if its absorbance value is less than 1. With consideration for the requirements and applicability, the maximum wavelength for Jatamansone solution was found using a UV-Visible spectrophotometer in full scan mode. The complete scan was processed using UV software, and the maximum was found using software. It was found that jatamansone has a wavelength of 278 nm (Fig. 2).

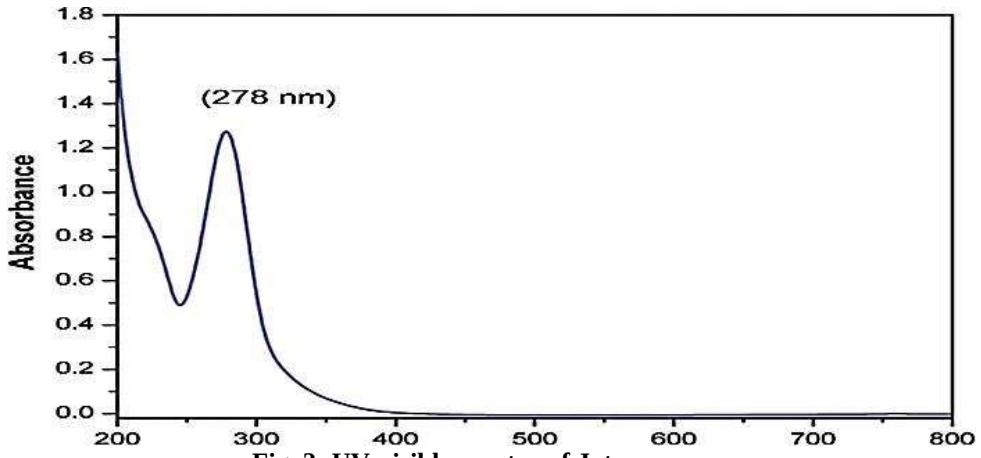

Fig. 2: UV-visible spectra of Jatamansone

Preparation of calibration curve:

An equation expressing the correlation between concentration and response is required for the quantification of unknown samples using a UV-visible spectrophotometer or any other experimental method of analysis. The calibration curve must be repeatable. Compared to the graphical method, the previously indicated approach is more universally accepted and repeatable. Considering the value of quantitative analysis of jatamansone, the calibration curve for the drug was constructed using six distinct calibration standards. A UV-Visible spectrophotometer was used to test the absorbance of multiple calibration standards at 278 nm in the fixed wavelength mode. Five iterations of the calibration curve were carried out, and Table 1 shows the mean values and variances.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 220-228 www.ijprajournal.com ISSN: 2456-4494

Table 1: Calibration standard data for Jatamansone

Concentration (µg/ml)	Absorbance
1	0.2561 ± 0.0015
2	0.4132 ± 0.0019
3	0.5598 ± 0.0014
4	0.7125 ± 0.0016
5	0.8752 ± 0.0012
6	0.9892±0.0011

Method validation Linearity and Range:

The two most important analytical technique parameters that show the bounds within which the planned approach should be applied for best results are linearity and range. Jatamansone was calibrated using a six-point curve that covered the range of 1-6µg/ml while accounting for range and linearity. The concentrations and related mean absorbance values are displayed in Table 1. As illustrated in Figure 3, the equation y = 0.1479x + 0.1149 was found when the calibration curve was subjected to least square regression analysis. The equation had a correlation value of 0.999.

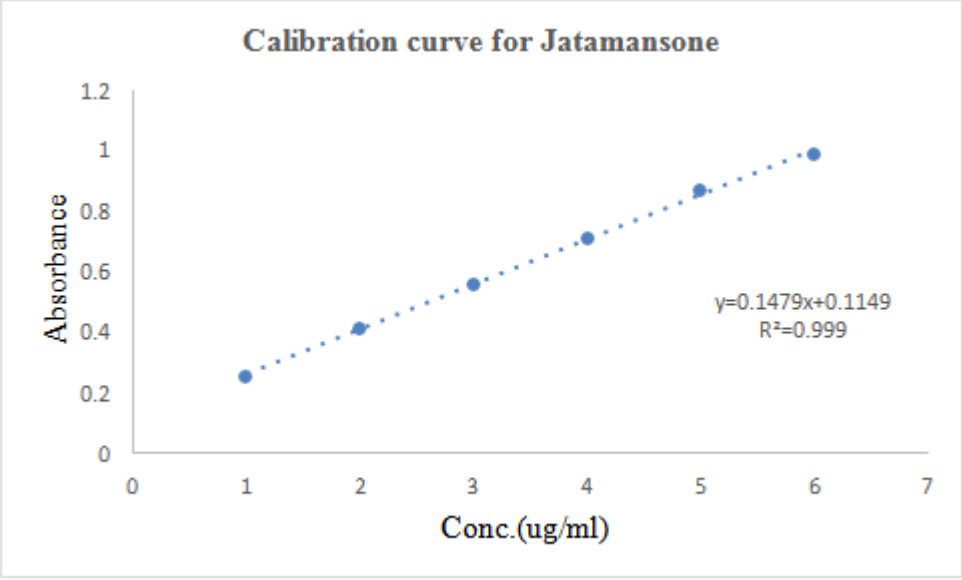

Fig. 3: Calibration curve for Jatamansone

According to linearity analysis, the developed UV method was found to be linear over the pre-defined concentration range of calibration standards.

Accuracy:

The degree to which the experimental value and the actual amount of the material in the matrix coincide is known as accuracy. For the results to be dependable at every stage of the determination process, accuracy must be ensured over the analytical method's whole calibration

International Journal of Pharmaceutical Research and Applications
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range. .. Recovery experiments were used to establish accuracy in the UV technique for jatamansone. The mean recovery of Jatamansone was determined to be 100.24% at an 80 percent standard addition, and 100.57% and 100.48% at 100 and 120 percent standard addition, respectively. Table 2 displays the percent RSD for the Jatamansone recovery studies, which was determined to be less than 2%.

Table 2: Accuracy data of UV method for Jatamansone

Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	1.5	1.2	99.45	100.24	0.521
80	1.5	1.2	101.07		
80	1.5	1.2	100.21		
100	3	3	100.85	100.57	0.759
100	3	3	101.12		
100	3	3	99.75		
120	5.5	6.6	99.36	100.48	1.119
120	5.5	6.6	101.23		
120	5.5	6.6	100.85		

The suggested UV approach was determined to be accurate, with a percent recovery of 98 to 102%, based on the results of accuracy experiments.

Precision:

The degree of scatter is a measure of precision. It conveys how repeatable the measurements are. The expectation is that an analytical process will yield repeatable outcomes. An exact analytical procedure yields accurate results. The developed UV technique's intra- and inter-day precision was determined at 1.5µg/ml, 3µg/ml, and 5.5µg/ml levels of jatamansone, considering the need for accurate but repeatable results. Tables 3 and 4 present the results of the intra- and inter-day precision investigations, respectively, in terms of mean absorbance values, percent assay, and percent RSD.

Table 3: Intra-day precision data of UV method for Jatamansone

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1.5	1.57	100.11	0.75	1.506	99.78	1.052	1.56	99.64	1.798
3	3.06	99.27	1.09	3.012	100.91	1.127	3.08	101.67	1.225
5.5	5.54	101.02	1.21	5.59	101.25	0.79	5.51	100.97	0.864

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 220-228 www.ijprajournal.com ISSN: 2456-4494

Table 4: Inter-day precision data of UV method for Jatamansone

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.505	99.97	0.97	1.55	99.61	1.572	1.562	98.72	1.256
3	3.019	100.24	1.561	3.026	100.26	0.889	3.01	100.26	0.982
5.5	5.56	98.86	0.892	5.507	98.33	1.223	5.53	99.74	1.132

Intra-day precision of proposed UV-Visible spectrophotometric method was found to be in the range of 0.75 to 1.798 whereas inter-day precision of the proposed method was found to be in between 0.889 to 1.572.

Robustness:

The capacity of an analytical method to maintain its performance in the face of minor, intentional changes to its parameters is known as its robustness. It is an essential analytical technique parameter since normal usage might result in small, inadvertent changes to method parameters like pH and solvent composition, which can hinder the method's effectiveness. It is anticipated that the performance of the analytical method should be unaffected by such a modification.It is expected that such a modification should have no effect on the analytical method's performance. As a result, a reliable analytical procedure can be chosen. Modifying the composition of the co-solvent solution showed the robustness of the suggested UV method. The method performance was unaffected by changing the methanol content in the co-solvent solution during slight change from 39-41%. Table 5 shows that the percent RSD values ranged between 0.856 and 1.461. The suggested UV technique is robust in nature, with percent RSD values below 2%.

Table 5: Robustness data of UV method for Jatamansone

Concentration (µg/ml)	**% MeOH:Water**	**Absorbance**	**% RSD**
3	40-60	0.5293	1.297
3	39-61	0.5798	0.856
3	41-59	0.5369	1.461

Ruggedness:

An analytical method's ruggedness is its capacity to withstand changes in external factors that affect its performance, such as modifications to labs, equipment, and analysts. Because they are unaffected by external or environmental factors, the rugged analytical method is used. The robustness of the suggested UV-visible approach was assessed using two distinct UV-visible spectrophotometers from two different labs on a jatamansone solution. After data processing and sample analysis, the % RSD values were discovered to range from 0.2248 to 0.9415. As indicated in Table 6, the proposed UV Visible spectrophotometric approach was proven to be robust, with percent RSD values less than 2%.

Table 6: Ruggedness data of UV method for Jatamansone

Concentration (µg/ml)	**Instruments**	**Absorbance**	**% RSD**
3	Jasco	0.6219	0.3457
3	Bioage	0.6354	0.5963
3	Analyst -I	0.6317	0.8729
3	Analyst -II	0.6422	0.9562

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Limit of Quantification (LOQ) and Limit of Detection (LOD):

The lowest concentration that can be examined with reasonable precision and accuracy is represented by LOQ. Table 7 displays the LOD and LOQ of the suggested UV technique, which were determined to be 0.3125 and 0.6369 µg/ml, respectively.

Table 7: LOD & LOQ data for UV method for Jatamansone

LOD	0.3125µg/ml
LOQ	0.6369µg/ml

The suggested method would be appropriate for assessing materials containing even minute amounts of jatamansone, according to the lower limit of quantification (LOQ) value.

Estimation of Jatamansone in Nardostachys jatamansi powder extracts:

Jatamansone content in Nardostachys jatamansi extracts was successfully estimated using the developed UV-Visible spectrophotometric method. In Soxhlet assisted extracts of Nardostachys jatamansi, concentration of Jatamansone was found to be 0.3059g /10gwhereas in ultrasound assisted extracts, the Jatamansone content was found to be 0.3065g/10g using the suggested UV – visible spectrophotometry method.

IV. CONCLUSION

It was successful to design a UV-visible spectrophotometric approach that is sensitive, accurate, and exact for estimating jatamansone. The method that was created proved to be resilient and strong, with the ability to determine the amount of Jatamansone present in various extracts of Nardostachys jatamansi.

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Conflict of Interest

Regarding the research authorship and/or publication of this paper, the author(s) have stated that they have no potential conflicts of interest.

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