International Journal of ChemTech Research
CODEN( USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 856-859, April-June 2010

# An UV-Visible Spectrophotometric Method for the Estimation of Plumbagin

**Shalini A. Israni, Nayana S. Kapadia, Suman. K. Lahiri, Gunvat K. Yadav, Mamta B. Shah***

**Department of Phytochemistry and Pharmacognosy, L.M. College of Pharmacy, Ahmedabad, Gujarat-380 009, India**

***Corres.autor: mbshah2007@ rediffmail.com, Contact No.: +91 9825766869***

**ABSTRACT:** A simple, rapid and sensitive spectrophotometric method for estimation of plumbagin in the roots of *P. rosea* is developed. The present investigation is further aimed at determination of the plumbagin content in the roots of different Plumbaginales namely, *Plumbago zeylanica*, *P. capensis*, *P. capensis var. alba* and *Vogelia indica* employing the same method. *V. indica* is widely used as an alternative drug to replace *P. rosea*. Studies in the past have reported the presence of plumbagin in *P. rosea*, *P. zeylanica* and *P. capensis*. Determination of plumbagin content in *P. capensis var. alba* and *V. indica* is reported in this work for the first time. Although, literature review details the application of HPTLC for estimation of plumbagin, the current work is an effort to develop an alternative method to determine the plumbagin content. The proposed spectrophotometric method is based on formation of magenta pink colored phenolate ion by reaction of naphthoquinones with alkalies. A graph of absorbance versus concentration shows that Beer's law is obeyed in the concentration range of 40µg/ml-240µg/ml. The pink colored chromogen is measured at maximum wavelength of 520nm. The results of analysis have been validated statistically and by recovery studies. The study revealed that the bioactive marker was found to be maximum in *P. rosea* (1.68%) followed by *P. zeylanica* (0.413%), *P. capensis var. alba* (0.33%), *P. capensis* (0.297%), and lastly *V. indica* (0.13%).

**Keywords:** Plumbagin, Plumbaginaceae, *Plumbago capensis*, *Plumbago capensis var. alba*, *Plumbago rosea*, *Plumbago zeylanica*, *Vogelia indica.*

## INTRODUCTION

The present study is an approach for quantification of plumbagin, a characteristic naphthoquinone present in the different species of *Plumbago* (Family: Plumbaginaceae), commonly known as Chitrak in India. Chitrak has long been used as a folk medicine in India and other countries for the treatment of various diseases e.g. cancer, rheumatoid arthritis, dysmenorrhoea, dyspepsia, leprosy etc. It is widely used as an abortifacient agent [1, 2]. Three species of genus *Plumbago* are recorded from India, namely, *P. rosea* Linn. (Rakta Chitrak), *P. zeylanica* Linn. (Sweta Chitrak) and *P. capensis* Thunb. (Kala Chitrak). Of these, the first two are considered medicinally important [3]. It is noted that, both *P. zeylanica* and *P. rosea* are popularly used as Chitrak without any concise specification, depending on the greater availability of the two, in different geographical regions of India. Moreover, many Ayurvedic formulations containing Chitrak do not give a clear distinction of the species used. Further, *Plumbago capensis var. alba* Hort. is also found to be growing as ornamental plant in India and *Vogelia indica* Gibson sharing the same family, is reported to be used as 'Red Chitrak' in Gujarat and certain regions of Rajasthan [4]. In light of this, different Plumbaginales have been quantitatively screened for the bioactive marker, plumbagin. In our current work, we report a suitable spectrophotometric method for estimation of plumbagin, which may further confirm the reliability of established HPTLC method [5]and vice versa. The crude samples were simultaneously evaluated by existing HPTLC method and proposed spectrophotometric method. The results were found to coincide for both the methods, assuring that spectrophotometric method is comparable with HPTLC method.

## MATERIALS AND METHODS

### Plant material

The fresh plants of *P. zeylanica*, *P. rosea*, *P. capensis* and *P. capensis var. alba* were procured from a reputed horticulture organization, Roses Corner located in Ahmedabad in the month of April 2003. The wildly growing fresh, well developed plants of *V. indica* were collected from remote areas of Jamnagar in the month of June 2003. The plants were authenticated by comparing the morphological characters described in the literature [6,7]. The authenticity of plants was further confirmed by a taxonomist of Gujarat Ayurved University, Jamnagar. Fresh roots of all the plants were cut into small pieces and dried at room temperature for 4-5 days. 60# powders of the dried roots, of all the five plants were prepared separately and stored in airtight glass bottles.

### Chemicals and reagents

All materials and reagents used were of analytical grade. Standard Stock solution of plumbagin was prepared in absolute alcohol having concentration of 1mg/ml.

### Isolation and identification of plumbagin

Plumbagin was isolated from the roots of *P. rosea* by the method of Shah et al. [8]

### Preparation of sample solution

5g of powder of each root drug was subjected to cold maceration using acetone (3x 50ml) separately. The pooled acetone extract was evaporated to dryness at room temperature and the residue was taken in 25ml chloroform, which was shaken with distilled water (3x 25ml) to remove water soluble impurities. The chloroform extract was evaporated to dark brown oily residue and was treated with 10ml of phosphoric acid (10%) for 30min. The yellow aqueous solution obtained was extracted with 5ml *n*-heptane. This sample aliquot was used for measuring absorbance after proper dilution to get desired range of concentration.

### Instrument

Hitachi U-2000 UV-Visible Spectrophotometer with 1cm matched quartz cells was used for all the spectral measurements.

### Determination of wavelength of maximum absorbance

To 1ml of standard stock solution (1mg/ml) of plumbagin, 1ml alcoholic KOH (10%) was added. The volume was then adjusted to the 5ml with absolute alcohol. The absorbance of the colored solution was scanned by Hitachi UV/Visible spectrophotometer in the range of 400 to 800 nm against reagent blank. The blank was prepared similarly in which volume of standard plumbagin was replaced by an equal volume of absolute alcohol. The maximum absorbance was obtained at 520nm.

### Optimization of reagent volume and determination of stability of colored product

Into a series of 5ml volumetric flask, standard stock solution of plumbagin (0.2ml) and reagent solution (0.4, 0.6, 0.8, 1.0, 1.2ml) were mixed thoroughly. The maximum absorbance was obtained with 1ml of 10% alcoholic KOH, which remained constant with increase in volume of reagent. The colored product (magenta pink) remained stable for 30 min.

### Lambert-Beer's curve for plumbagin

In a series of 5ml volumetric flask, 0.2, 0.3, 0.4, 0.8 and 1.2ml of standard stock solution of plumbagin were mixed with 1ml of 10% alcoholic KOH and volume was made up to the mark with absolute alcohol. The absorbance of colored solution was measured at 520nm against reagent blank. The absorbance was plotted against concentration of plumbagin and the concentration of unknown solution was computed from the calibration graph or from the regression equation (Fig 1, Table 1).

**Table 1. Calibration data of standard plumbagin.**

| Concentration of Plumbagin (µg/ml) | Absorbance at 520nm Mean ± S.D. | % C.V. |
|---|---|---|
| 40 | 0.037 ± 0.0013 | 3.5 |
| 60 | 0.108 ± 0.0024 | 2.2 |
| 80 | 0.172 ± 0.004 | 2.3 |
| 160 | 0.413 ± 0.0083 | 2.0 |
| 240 | 0.610 ± 0.0199 | 3.2 |

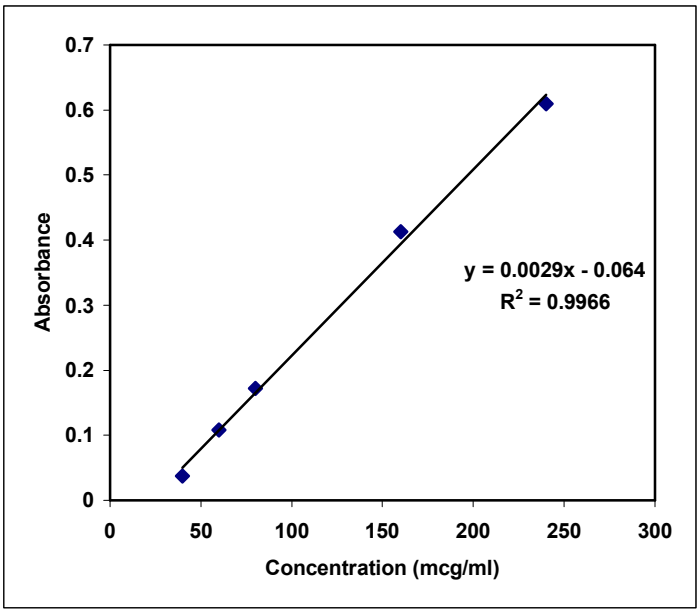


**Figure 1. Calibration curve of plumbagin.**

## RESULTS AND DISCUSSION

The samples obeyed Beer's law in the range between 40-240µg/ml. The linear regression obtained is Y= 0.0029x-0.064. The correlation coefficient was found to be 0.9966. For estimation of plumbagin, sample solutions were diluted prior to absorbance measurement. In case of *P. zeylanica*, *P. capensis*, and *P. capensis var. alba*, 0.2ml of sample solution as prepared by above method was diluted to 5ml with absolute alcohol, while in case of *P. rosea*, 0.1ml was diluted to 10ml and for *V. indica*, 1ml was diluted to 5ml. In each case, 1ml of aliquot was used for measurement of absorbance. The percentage of plumbagin in all the samples calculated from the calibration curve is shown in Table 2. The plumbagin content found in *P. rosea* was maximum (1.7%), followed by 0.39% *P. zeylanica,* 0.3% in *P. capensis var. alba,* 0.27% in *P. capensis and* 0.15%in *V. indica*. The accuracy of results of estimation was tested further by the recovery experiment that indicated the absence of interference of reagents. The method was validated and the results obtained are shown in Table 3. Limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were also calculated.

The present study offers a reliable new method for estimation of plumbagin. The method may serve as a better proposition in case of lack of facilities of more sophisticated instruments such as HPTLC. The results of the study indicate a significant difference in concentration of plumbagin in *P. rosea* and other Plumbaginales. *P. rosea* was found to contain maximum plumbagin, whereas the other species of *Plumbago* contained almost similar amount of plumbagin. *V. indica* which is widely replaced for *P. rosea* was also found to contain plumbagin, which is considerably less in comparison to *P. rosea.* It can be interpreted that *P. rosea* having maximum amount of plumbagin should be used in formulations, but as the plant is found in cultivated condition and not as commonly seen as *P. zeylanica*, the later is more frequently used. Although the most research has been focused on the two parent species of *Plumbago* (*P. rosea* and *P. zeylanica)*, our studies suggest that *P. capensis* and *P. capensis var. alba* should also be given due attention since, they also contained appreciable quantities of plumbagin. Presence of plumbagin in *V. indica* directs its use as a substitute of *P. rosea*.

***Table 2.*** **Estimation of plumbagin in different species of *Plumbago* and *Vogelia indica.***

| Samples | Absorbance | Average % of Plumbagin ± S. D. | % C.V. |
|---|---|---|---|
| ***P. zeylanica*** | 0.416 | 0.413 ± 0.005 | 1.2 |
| ***P. rosea*** | 0.424 | 1.68 ± 0.0072 | 1.6 |
| ***P. capensis*** | 0.281 | 0.297 ± 0.002 | 0.8 |
| ***P. capensis var. alba*** | 0.321 | 0.33 ± 0.0088 | 2.6 |
| ***V. indica*** | 0.692 | 0.13 ± 0.0098 | 1.4 |

***Table 3.*** **Summary of validation parameters.**

| Parameters | Results |
|---|---|
| Absorbance maxima | 520nm |
| LOD | 20µg/ml |
| LOQ | 40µg/ml |
| Linearity range | 40- 240µg/ml |
| Correlation coefficient | 0.9966 |
| Precision (%CV) | |
| Intraday | 2.3-0.24 |
| Interday | 2.0-3.5 |
| % Recovery (Accuracy) | 98.0-99.41 |

**REFERENCES**


1. Masataka I., Kazumi T., Hiroshi F., Cardiotonic action of plumbagin on guinea pig papillary muscle. *Planta Medica,*1991; *57*: 317-319.
2. Watt G., *A Dictionary of the Economic Products of India*, Part-I, Vol. VI. Delhi, India, Periodical Experts, 1972; 294-296.
3. Warrier P.K., Nambiar V.P.K., Ramankutty C., (Eds.) *Indian Medicinal Plants, A Compendium of 500 Species*, Vol. IV., Madras, India, Orient Longman Ltd., 1997; 321.
4. Vaidya B.G., *Some Controversial Drugs in Indian Medicine,* Delhi, India, Chaukambha Orientalia, 1982;182-184.
5. Gupta A.P., Verma R.K., Gupta M.M., Sushil Kumar (1999): Estimation of plumbagin using HPTLC. *Journal of Medicinal and Aromatic Plant Sciences,* 1999; *21(3)*: 661-663.
6. Drury H., In: Singh B, Singh PM, eds., *Handbook of The Indian Flora*. Dehradun, India, 1982; 575.
7. Kirtikar K.R., Basu B.D., *Indian Medicinal Plants*, 2nd ed., Vol. III. Delhi, India, M/S Periodical Experts, 1975; 1465-1471.
8. Shah M.B., Israni S.A., Kapadia N.S., A simple method of isolation of plumbagin from *P. rosea*. *Pharmaceutical Biology,* 2005; *23*: 551-553.


*****