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HPTLC ANALYSIS OF BERBERINE IN *ARGEMONE MEXICANA* L

Pradeep Kumar Samal*

SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, C.G, India

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ABSTRACT

***Argemone mexicana* (Mexican poppy) is a species of poppy found in Mexico and now widely naturalized in many parts of the world. It is also widely available in tribal belt of Chhattisgarh. Literature survey reveals that it contains 22–36% of a pale yellow non-edible oil, called argemone oil or katkar oil, which contains the toxic alkaloids sanguinarine and dihydrosanguinarine. Four quaternary isoquinoline alkaloids, dehydrocorydalmine, jatrorrhizine, columbamine, and oxyberberine, have been isolated from the whole plant. Berberine, an isoquinoline alkaloid isolated from several herbs including, *Argemone mexicana* has a long history of use in various disorders. So an attempt has been taken for the quantification of berberine in *Argemone mexicana* available in Chhattisgarh. HPTLC was the method of choice. the mobile phase consisting of toluene: ethyl acetate (9:3, v/v) provided a sharp and well defined peak at RF value 0.42. LOD (0.120µg) and LOQ (0.362 µg) suggests high efficiency of the process. Accuracy in terms of % recovery (98.19±1.204) which shows high extraction efficiency of berberine from formulation components. The HPTLC method developed here for the quantification of berberine in A. Mexicana is simple, rapid, cost-effective and easily adaptable for screening and quantitative determination than any other analytical technique.**

Key words: *Argemone mexicana,* Berberine, HPTLC, Precision, Accuracy.

INTRODUCTION:

Argemone mexicana (Mexican poppy) is a species of poppy found in Mexico and now widely naturalized in many parts of the world. It is also widely available in tribal belt of Chhattisgarh. Various parts of the plant or the plant as a whole is used as treatment of so many disorders by traditional practitioners. An infusion is made to relieve kidney pain, to help expel a torn placenta, and in general to help cleanse the body after parturition. Also decoction of this plant is used as a sedative and analgesic tea, including for use to help alleviate migraine headaches. The seeds are taken as a laxative[1]. Further it is used by traditional healers in Mali to treat malaria. Literature survey reveals that it contains 22–36% of a pale yellow non-edible oil, called argemone oil or katkar oil, which contains the toxic alkaloids sanguinarine and dihydrosanguinarine. Four quaternary isoquinoline alkaloids, dehydrocorydalmine, jatrorrhizine, columbamine, and oxyberberine, have been isolated from the whole plant[2]. Berberine, an isoquinoline alkaloid isolated from several herbs including, *Argemone mexicana* has a long history of use in various disorders. As a traditional medicine or dietary supplement, berberine has shown some activity against fungal infections, *Candida albicans*, yeast, parasites, and bacterial/viral infections[3-4]. Berberine seems to exert synergistic effects with fluconazole even in drug-resistant C. albicans infections[5]. Some research has been undertaken into possible use against MRSA infection[6]. Berberine is considered antibiotic[7-8] When applied in vitro and in combination with methoxyhydnocarpin, an inhibitor of multidrug resistance pumps, berberine inhibits growth of *Staphylococcus aureus*[9] and Microcystis aeruginosa[10] a toxic cyanobacterium. Berberine is a component of some eye drop formulations. There is some evidence it is useful in the treatment of trachoma and it has been a standard treatment for leishmaniasis[11]. Berberine prevents and

Address for correspondence

Pradeep Kumar Samal

Email- samalpharmacology@rediffmail.com

suppresses proinflammatory cytokines, E-selectin, and genes, and increases adiponectin expression which partly explains its versatile health effects. Berberine is a nucleic acid-binding isoquinolone alkaloid with wide potential therapeutic properties. So an attempt has been taken for the quantification of berberine in *Argemone mexicana* available in Chhattisgarh. HPTLC was the method of choice because several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC. It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions[12].

MATERIAL AND METHOD:

Chemicals:

All the chemicals, including solvents, were of analytical grade from Sigma Aldrich, India. Berberine, was obtained from the Sigmae Aldrich , India. The HPTLC plate silica gel 60F254 (20cm×20cm) was purchased from E.Merck, Darstadt, Germany, supplied by anchrom technologies, Mumbai.

Preparation of extract for analysis of Berberine:

Dried whole plant powder of *Argemone mexicana* L. was extracted with methanol with the help of soxhlet apparatus. The obtained extract was dried, the extractive value was found to be 9.81 % w/w. A known concentration of the extract was prepared in methanol and filtered. The filtered solution was applied on TLC plate followed by development and scanning for the determination of berberine. Analysis was repeated in triplicate.

Preparation of standard solution:

Berberine solution of strength 1µg/µl was prepared in methanol. Different amounts 2,4,6,8 and 10 µl of standard was spotted in triplicate on TLC plate, using Camag Linomat V sample applicator (Camag, Muttenz, Switzerland) and a 100µl Hamilton syringe for preparing five point calibration curve.

HPTLC instrumentation:

The HPTLC system (Camag, Muttanz, Switzerland) consisted of (i) TLC scanner connected to a PC running WinCATS software under MS Office; (ii) Linomat V sample applicator using 100µl syringes and connected to a nitrogen tank. Each plate accommodated 8 tracks of samples and standards, applied according to following settings: band width 5 mm; distance between bands 10 mm; application volume 2-10 µl; gas flow 150 nl/s. The plates were developed to 7 cm in a twin trough glass chamber presaturated with the upper layer of mixture toluene: ethyl acetate (9:3, v/v). The developed plates were dried. The scanner was set for maximum light optimization and with the following settings: slit dimension, 5.00 mm × 0.45 mm, micro; scanning speed, 20 mm/s; data resolution, 100µm/step; scanning wave length, 266 nm in absorbance reflectance mode. All remaining measurement parameters were left at default settings.

Development of the optimum mobile phase:

The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity.

Calibration plot of eucalyptol:

Berberine standard solution of 2,4,6,8 and 10 µl were spotted in triplicate on TLC plate to obtain Concentrations of 2,4,6,8 and 10 µg $spot^{-1}$ of berberine, respectively. The data of peak area versus drug concentration were treated by linear least-square regression.

Purity of Spot in chromatogram:

The spot obtained on the chromatogram were analysed at wavelength 266 nm at three points in the standard as well as in sample i.e. in the point start to middle, middle and finally in the middle to end.

Validation of the method:

Linearity, limits of detection and quantification:

The linearity of the detector response for the prepared standards was assessed by means of linear regression regarding the amounts of each standard, measured in µg, and the area of the corresponding peak on the chromatogram (n=4). Linearity was also

confirmed for '*Argenone mexicana*' extract. After chromatographic separation, the peak areas obtained were plotted against the extract concentrations by linear regression. Limits of detection and quantification were determined by calculation of the signal to noise ratio. Signal-to-noise ratios of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit, respectively, of the method.

Accuracy:

Recovery studies were carried out to check accuracy of the method. Recovery experiments were Performed by adding three different amounts of berberine i.e., 25, 50 and 75% of the amount of berberine analysed from different formulations and result was analysed (n=6).

Precision:

The intra-day precision was evaluated by analysing berberine repeatedly at concentration range of 5-50 µg/spot (n=5). The inter-day precision was evaluated by analysing berberine at concentration range of 5-50 µg/spot over a period of 10 days (n=5).

RESULT AND DISCUSSION:

The mobile phase toluene: ethyl acetate (9:2, v/v) provided good resolution with RF value 0.25 for berberine but typical peak nature was missing. Finally, the mobile phase consisting of toluene: ethyl acetate (9:3, v/v) provided a sharp and well defined peak at RF value 0.42. The well defined spot was obtained when the chamber was saturated with the mobile phase for 15 min at room temperature. Berberine showed a good linear relationship over the concentration range 2-10µg per spot with respect to peak area (n=3). The linearity was observed with the regression coefficient being 0.9989 with Standard error of the mean (SEM) of 0.0028. No significant differences were observed in the slopes of standard curve. Purity of each spot which is scanned at wave length 266nm with value of r (S, M) within the range, 0.997-0.998 and r (M, E) within the range, 0.999-0.997. LOD, LOQ, accuracy and precision were evaluated for quantitative purposes. LOD (0.120µg) and LOQ (0.362 µg) suggests high efficiency of the process. Accuracy in terms of % recovery (98.19±1.204) which shows high extraction efficiency of berberine from formulation components. The % coefficient of variance for intra-day and inter-day precision was found to be 3.68 and 6.42 respectively which is comparable and within the limits. Hence, the proposed method can be used for estimation of berberine in *A. mexicana*. The berberine content in *A. Mexicana* was found to be 4.12±0.21µg /g of plant powder. The proposed HPTLC technique is found to be precise and accurate. Further, the method is sensitive for the analysis of berberine in pharmaceutical formulations. With the growing demand of herbal drugs in the herbal drug market and with the increased belief in the usage of green medicine (herbal drugs), this standardization tool will help in maintaining the quality and batch to batch consistency of this important plant.

CONCLUSION:

The HPTLC method developed here for the quantification of berberine in A. Mexicana is simple, rapid, cost-effective and easily adaptable for screening and quantitative determination than any other analytical technique.

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