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Quantitative estimation of wedelolactone in *Eclipta alba* hask using high performance liquid chromatography

Purnima Hamrapurkar*, Manasi Chachad, Mitesh Phale

Department of Pharmaceutical Analysis, Prin. K.M.Kundnani College of Pharmacy,

Jote Joy Building, Rambhau Salgaonkar Marg, Cuffe Parade, Colaba, Mumbai-400005, (INDIA)

Tel : +91 22 2216 4368; Fax : +91 22 22165282

E-mail : phamrapurkar@gmail.com

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ABSTRACT

Wedelolactone is a characterizing compound present in the plant *Eclipta alba*, used in viral hepatitis, liver diseases, skin and hair care. A simple, accurate and sensitive HPLC method was developed for estimation of wedelolactone, a furanocoumarin present as a major active constituent in the plant *Eclipta alba*. The chromatographic separation was carried out on RP C18 (Waters) column (250×4.6 mm, 10µm) using an isocratic mobile phase, Acetonitrile: Water (35:65 % v/v) pumped at a flow rate of 1ml/min. The eluent was monitored at 351 nm. The method is specific and linear over the range of 300 ng/ml to 1500 ng/ml. The method was statistically validated for precision, accuracy, robustness and recovery. The HPLC method can be applied for identification and quantitation of wedelolactone in herbal extracts of *Eclipta alba*.

KEYWORDS

Wedelolactone;
HPLC;
Quantitative estimation.

INTRODUCTION

Eclipta alba Hask commonly known as Bhringaraj is an annual herb grown throughout India in moist and dump land. *E.alba* has a short, flat or round stem, deep brown in color belonging to family Asteraceae. The plant is widely used in viral hepatitis, liver diseases, skin and hair care, anemia, dysentery, eye disease, asthma and liver cirrhosis. Thus it forms an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation[1,2]. Eclipta alba leaves showed antihyperglycemic activity[3]. The roots of *E.alba* were found effective in wound healing[4]. The genus *Eclipta* shows the presence of wedelolactone, demethylwedelolactone, ecliptal, hentriacontanol, and aclabosaponinsI-IV phytoconstituents. But wedelolac tone is the major compound responsible for the hepato protective activity[5].

Literature survey revealed that HPLC[5-8], HPTLC [9,10] and UV spectrophotometry[11], spectrofluorometric[12] methods had been reported for the estimation of wedelolactone in the herbal extract. However these methods suffer from drawback such as the poor resolution, long retention time and sensitivity. Thus, an attempt has also been made to develop and validate HPLC method for the analysis of wedelolactone which would be highly sensitive, having good resolution, shorter retention time and reproducible.

EXPERIMENTAL

Chemicals and reagents

The wedelolactone standard was obtained from Natural Remedies Pvt. Ltd., Bangalore, India and char-

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acterized by Ultra-violet (UV), Infrared, Proton nuclear magnetic resonance and by mass spectroscopy to confirm their identity and purity. HPLC grade Acetonitrile and Methanol was purchased from Merck (Darmstadt, Germany) and acetic acid of AR (Analytical Reagent) grade was procured form Qualigens Fine Chemicals, Mumbai, India. Deionised and ultra pure water used in all experiment was obtained from Milli-Q system (Millipore, USA).

Plant material

The aerial plant of *Eclipta alba* Hask was procured from Mumbai, and authenticated at Agharkar Research Institute, Pune, India. (Certificate dated 12/02/07)

Equipment

pH of the mobile phase was checked on a pH/ion analyzer (Lab India PHAN, India). The HPLC system employed in the method development and validation was Jasco PU 1580 intelligent HPLC pump with a Rheodyne 7725 loop injector, Jasco UV-Vis 1520 detector and Borwin Chromatographic Software (version 1.21) as data integrator.

Optimized Chromatographic Conditions

The chromatographic separation was achieved on RP Spherisorb C-18 (Waters) column (250×4.6mm, 10μm), using a mobile phase consisting a mixture of Acetonitrile: Water (35:65 % v/v). The pH of the mobile phase was adjusted to 3.2 with acetic acid. All reagents were filtered through 0.45-μm filter paper and sonicated before use. The injection volume was 100 μl. The UV-Vis detector was set at of wavelength 351nm. The experiment was performed at room temperature and the flow was fixed at 1.0 ml/min.

Preparation of standard solution

A stock solution of wedelolactone (1mg/ml) was prepared in methanol from the standard obtained. Standard solutions were prepared by dilution of the stock solution with mobile phase to give solution in concentration range of 300 ng/ml to 1500 ng/ml.

Sample preparation

Soxhlet method for extraction

The powdered plant material was extracted with methanol using Soxhlet apparatus. The extract obtained was then diluted appropriately with methanol.

Supercritical fluid extraction

1.0g of powdered plant material was weighed and transferred to the SFE vessel. The optimized conditions of SFE are as follows Gas-CO_2, Pressure-150 kg/cm^2, Temperature- 45^{0}C, Modifier-methanol, Flow rate-0.2 ml/min, CO_2 flow rate- 2.5ml/min, extraction time- 30 minutes. The extract was then concentrated under vaccum and diluted with methanol.

Isolation of wedelolactone

Isolation of Wedelolactone was carried out using column chromatography and characterization was done using various spectral studies.

Validation of method

A stock solution of the drug was prepared at strength of 1mg/ml. It was diluted to prepare solutions containing 300 ng/ml to 1500 ng/ml of wedelolactone. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (100μl)

Twelve injections, of three different concentrations (300, 700 and 1500 ng/ml), were given on the same day and the values of relative standard deviation (R.S.D.) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Accuracy was evaluated for the known concentration of the drug. The recovery of the added drug was determined. The specificity of the method was ascertained by analyzing standard wedelolactone and then comparing the sample retention time (RT) of wedelolactone in herbal extract with the RT of the standard.

The LOD and LOQ were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution was recorded. The flow rate of the mobile phase was 1.0 ml/min.

The solution stability was carried out by leaving both the test solution of sample and standard in tightly capped volumetric flasks at -20^{0}C for 7 days. The sample solution was assayed after 7 days against fresh samples.

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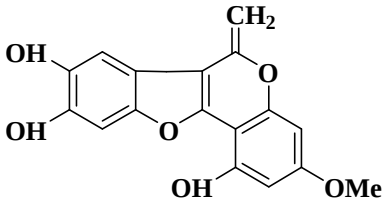

Figure 1 : Chemical structure of wedelolactone

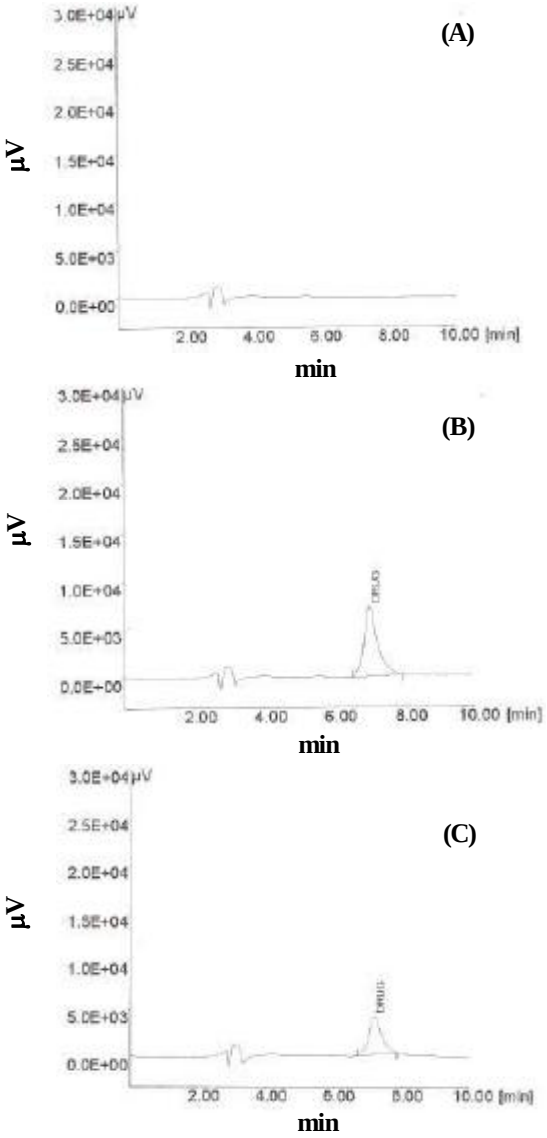

Figure 2: HPLC chromatogram of: (A) Blank, (B) wedelolactone standard (C) *Eclipta alba* extract

RESULTS AND DISCUSSION

Wedelolactone a furanocoumarin, insoluble in water and soluble in methanol. Hence methanol was used for the extraction of Wedelolactone using classical method and supercritical fluid extraction technique. The yield of phyllanthin by soxhlet and SFE technique were found to be 0.79 % and 0.88 % and time taken for extraction was 6.0 h and 0.5 h respectively. Considering the chemical nature and polarity of Wedelolactone longer chain alkyl bonded column such as C18 was tried with various compositions of mobile phases. Initially methanol and water used as a mobile phase but as Wedelolactone is slightly semipolar in nature it was not eluted properly in methanol and gave broad peak and long retention time hence it was replaced with less polar solvent acetonitrile which reduce the interaction between silanol group of stationary phase and Wedelolactone results in sharp peak and shorter retention time. A mobile phase comprising of acetonitrile: water (35:65 % v/v) was found to be optimum and detection was carried out using UV detector at 351 nm.

TABLE 1: System suitability test parameters

System suitability parameters	Values
Retention time (min)	7.5
Tailing factor (asymmetric factor)	1.25

TABLE 2: Summary of validation parameters for the proposed method

Parameters	Values
Detection limit	100 ng/ml
Quantitation limit	300 ng/ml
Calibration range	300-1000 ng/ml
Accuracy (%)	96.23-97.80
Precision (RSD, %)	
Intraday (n=6)	0.71-1.89
Interday (n=6)	0.82-1.87
Correlation coefficient (r)	0.999

The method was validated with respect to parameters like linearity, precision, accuracy, specificity and robustness.

LOD and LOQ were found to be 100 ng/ml and 300 ng/ml respectively. The method was found to be linear over the range of 300 ng/ml to 1500 ng/ml with regression value 0.999.

TABLE 2 provides data obtained from the precision experiments. The R.S.D. values for intra- and inter-day precision were <5 and 4 %, respectively, thereby indicating that the method was sufficiently precise. The method was found to be specific to the drug. The drug peak was free from any coeluting peak. The result indicated that the method was capable with high precision.

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The % R.S.D. of the assay of wedelolactone during solution stability experiments were within 2%. No significant changes were observed during solution stability. The solution stability data confirms that the sample solutions were stable at least for 7 days.

The validated method was then successfully applied for the estimation of Wedelolactone from various extracts obtained by soxhlet apparatus and supercritical fluid extraction technique. The results of SFE showed improved extraction efficiency and reduced extraction time.

CONCLUSION

The developed method was found to be specific, accurate, simple, precise and reproducible and hence can be used for routine analysis of extract of *Eclipta alba* for wedelolactone content.

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