



Estimation of wedelolactone and demethylwedelolactone in *Eclipta alba* Hassk. by improved chromatographic analysis

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Abstract

Objective: Estimation of wedelolactone (WDL) and demethylwedelolactone (DWDL) in *Eclipta alba* Hassk. by HPLC. **Materials and methods:** An Isocratic, reverse phase (RP) HPLC procedure for the standardization of *Eclipta alba* using a mixture of 0.1% v/v Ortho phosphoric acid and acetonitrile as mobile phase C8 column as stationary phase and photo diode array detector. **Results:** The above method shows high resolution, accuracy, economical and reproducibility. **Conclusions:** The method developed is useful in standardization of *Eclipta alba* material.

Key words: *Eclipta alba*, wedelolactone, demethylwedelolactone, HPLC.

1. Introduction

Eclipta alba (L.) Hassk. (synonym: *Eclipta prostrata* L. "bhringaraja") belongs to family Asteraceae is a small, branched annual herb with white flower heads inhabiting tropical and subtropical regions of the world. The plant grows abundantly on a weed in cool, moist places all over India [1]. In Ayurvedic medicine, *Eclipta alba* is said to be the best drug for the treatment of liver cirrhosis and infective hepatitis and coumestans (WDL and DWDL) have been reported as main active

principles [2-3]. Wagner *et al.* [3] reported the estimation of these 2 bioactive compounds by HPLC (gradient method). The present investigation was carried out to estimate these active principles by an improved HPLC method.

2. Materials and method

2.1 Plant material

After taxonomic verification, fresh plants of *Eclipta alba* Hassk. were collected in the month of June 2000 from the department of

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Horticulture (Medicinal and Aromatic Plants), GKVK, UAS, Bangalore. The whole plant were dried carefully in a vacuum oven below 65°C for 48 h. Voucher samples were deposited at pharmacognosy department of Natural Remedies Pvt. Ltd., Bangalore.

2.2 Chemicals

Acetonitrile – Ranbaxy (HPLC grade),
Methanol - Ranbaxy (HPLC grade),
Orthophosphoric acid –Ranbaxy (AR grade).

2.3 Extraction of Plant Material

2.5 g of finely powdered *Eclipta alba* whole plant was extracted with HPLC grade methanol (25 x 6 times) and concentrated to 100 ml.

2.4 Chromatographic system

Shimadzu HPLC equipped with LC 8A pump, SPD-M 10 AVP photodiode array detector in combination with class LC 10 software, column: C8- octyl silane, 250 x 4.6 mm, 0.5 µ size (Merck), mobile phase: 0.1% v/v Ortho phosphoric acid in water and acetonitrile (75:25). Flow rate: 1 ml/min, injection volume: 20 µl, Detection: 254 nm.

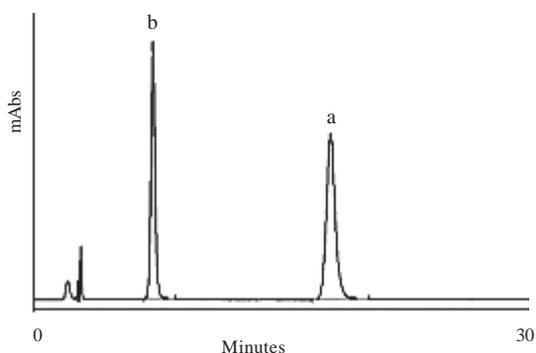


Figure 1. HPLC chromatogram of (a) wedelolactone (b) demethylwedelolactone

Table 1.

WDL and DWDL content in *Eclipta alba*

Sample	WDL	DWDL
1	0.08	0.01
2	0.09	0.015
3	0.070	0.009

Values are in % w/w

2.5 Estimation of WDL and DWDL of sample and recovery studies

Determination of WDL and DWDL were done by injecting separately 20 µl of 200 µg/ml respective methanolic solution and retention time was identified. Calibration was done by injecting 20 µl of different concentration of each solution in triplicate, average area was calculated.

Estimation was carried out by injecting 20 µl of each sample solution (refer 2.3), percentage was calculated using area under curve, which is compared with standards. The accuracy of estimation was validated using recovery studies.

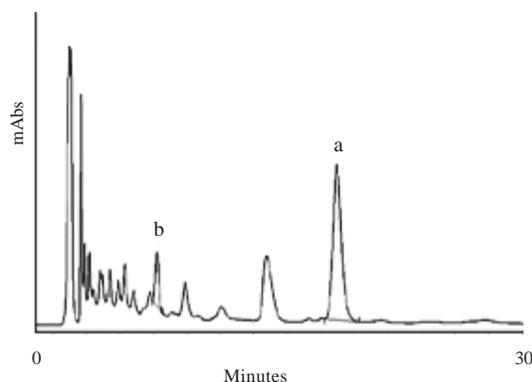


Figure 2. HPLC chromatogram of (a) wedelolactone (b) demethylwedelolactone in *Eclipta alba* Hassk.

3. Results and discussion

The goal of this investigation was to develop fast, selective, simple, accurate with high resolution HPLC method for estimation of WDL and DWDL from *Eclipta alba*, which has been achieved and confirmed by validation parameters like specificity, linearity, accuracy, precision, repeatability and reproducibility and system sensitivity.

A simple sample preparation with a methanolic extraction makes the method effective, accurate

and economic for the purpose of quality control. Concerning the validation data obtained, the method developed is suitable for the estimation of both WDL and DWDL. The linearity was ensured over a wide concentration range. The repeatability and the intermediate precision were sufficient as well. The earlier reported method [3] adopted gradient separation, whereas the current method adopts isocratic separation. The present method has been found to be relatively easy and accurate.

References

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