

**A RAPID DENSITOMETRIC METHOD FOR QUANTIFICATION OF WEDELOLACTONE IN HERBAL FORMULATIONS USING HPTLC**

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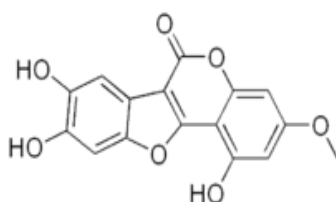
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**ABSTRACT:** Wedelolactone possesses a wide range of biological activities and is used for the treatment of hepatitis, cirrhosis. A simple HPTLC method has been developed for the quantification of wedelolactone. The samples were dissolved in methanol and linear ascending development was carried out in twin trough glass chamber saturated with mobile phase consisting of toluene: ethyl acetate: acetone: formic acid (6:2:1:1v/v/v/v). Spectrodensitometric scanning was performed by TLC scanner III (CAMAG) in absorbance mode at the wavelength of 351nm. The system was found to give compact spots for wedelolactone (R<sub>f</sub> value of 0.39 ± 0.03). The linear regression analysis data for the calibration plots showed good linear relationship with r<sup>2</sup> = 0.9963 in the concentration range 400-800 ng/spot with respect to peak area. According to the ICH guidelines the method was validated for accuracy, precision, recovery, and robustness. The wedelolactone content quantified from herbal formulations was found well within limits. Statistical analysis of the data showed that the method is reproducible and selective for the estimation of wedelolactone.

**Key words:** HPTLC; Wedelolactone; Herbal formulations.

## INTRODUCTION

*Eclipta alba* (Asteraceae) is traditional medicinal plant known as bhringaraj. The plant is an active ingredient of many herbal formulation prescribed for liver ailments and shows effect on liver cell generation [The Wealth of India, 2002]. This plant has been well reported to exhibit hepatoprotective activity [Ma-Ma et.al,1978] and in the treatment of infective hepatitis [Dixit and Achar, 1979]. The leaves of *Eclipta alba* showed antihyperglycemic activity [Ananthi, et.al, 2003]. A number of compounds had been isolated from the plant. Wedelolactone and de-methyl wedelolactone, complex furanocomarins have been identified as its hepatoprotective principles [Wagner, et.al., 1986]. Literature survey revealed that HPLC [Wagner, et.al., 1986] and UV spectrophotometry [Das, et.al.,1990] methods had been reported for the estimation of wedelolactone (Figure.1) in a methanol extract. Characterization of bhringaraj herb by ICP-MS analysis, optical absorption, infrared and EPR spectroscopic methods also reported [Reddy, et.al., 2008]. In the present paper, an accurate HPTLC method for quantitative determination of wedelolactone in herbal formulations is described. The proposed method was validated in compliance with ICH guidelines [Ansari, et.al., 2005].



**Figure.1. Chemical structure of wedelolactone.**

## 2. Experimental

### 2.1 Herbal Materials and Chemicals

Reference standard wedelolactone was obtained from Sigma-Aldrich Chemicals Pvt., New Delhi, India. Analytical grade reagents: toluene, ethyl acetate, acetone, formic acid and methanol (Merck Chemicals, India) were used. Herbal formulations of bhringaraj Ghanvati from different manufacturers (coded as Formulation 1 and Formulation 2) were procured from the local market.

#### *Standard and sample solutions*

Wedelolactone (10 mg) was dissolved into a 10 ml of methanol. This solution termed as stock solution of standard Wedelolactone ( $1 \text{ mg mL}^{-1}$  or  $1000 \text{ } \mu\text{g mL}^{-1}$ ). In both Bhringaraj Ghanavati Formulations 1 and 2 were contain aqueous extract of Bhringaraj 250 mg. Weighed Separately 10 Ghanavati from Formulation 1 and Formulation 2, crushed separately in two different glass crucibles to get fine powder of each formulation. Dissolve all contents of each Ghanavati formulation (10 Ghanavati) separately in sufficient quantity of methanol and adjusted the final volume to 25 mL with the same, to get two solutions of 100mg/ml. These solutions used for further analysis.

## 2.2 Chromatography

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60<sub>F</sub>-254 plates, [20 cm × 10 cm with 250 μm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 °C for 5 min prior to chromatography. A constant application rate of 0.1 μL/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: ethyl acetate: acetone: formic acid (6: 2:1:1 v/v/v/v) and 15 ml of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 °C ± 2) at relative humidity of 60 % ± 5. The length of each chromatogram run was 9 cm. Following the development the TLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 351 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. The amount of wedelolactone was computed from peak areas.

## 2.3 Calibration Curve

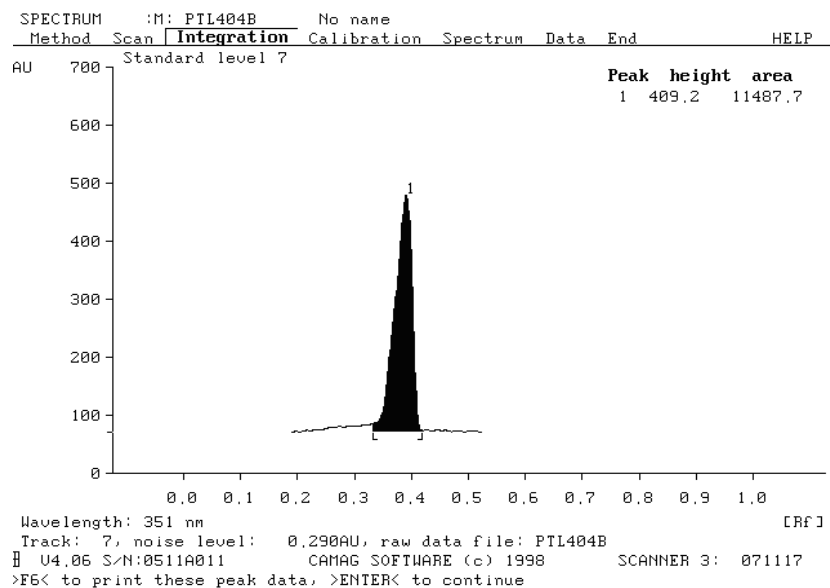
Standard solution of wedelolactone concentration of 100 μg mL<sup>-1</sup> prepared and 4, 5, 6, 7 and 8 μl from standard solution applied in triplicate on TLC plate to obtain final concentration 400-800 ng/spot. The plate was then developed using the optimized mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves. [Q2A, ICH, Q2 (R1), 2005].

## 3. RESULTS AND DISCUSSION

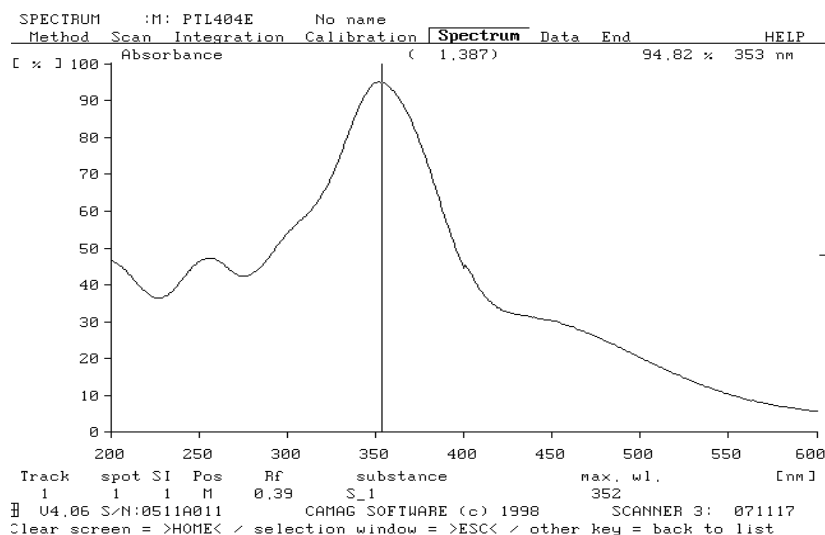
### 3.1 Mobile Phase Development

The mixtures of several mobile phases were tried to separate spot of wedelolactone from other spots and get stable peak.

The solvent system used was toluene: ethyl acetate: acetone: formic acid (6:2:1:1) was selected for estimation of wedelolactone, which gave good resolution. Good chromatogram in (Figure.2) was attained with Rf value  $0.39 \pm 0.03$ . The absorption spectrum of wedelolactone is shown in (Figure.3). The wavelength 351 nm was used for quantification of sample.



**Figure.2. Chromatogram of Wedelolactone (600 ng spot<sup>-1</sup>): peak 1 (Rf =  $0.39 \pm 0.03$ ), mobile phase: toluene: ethyl acetate: acetone: formic acid (6:2:1:1, v/v/v/v).**



**Fig.3. Absorption spectra of Wedelolactone.**

### 3.2 Method validation

#### 3.2.1 Specificity

The specificity of method was ascertained by standard wedelolactone and samples (Formulation 1 and Formulation 2). The spots of standard wedelolactone and samples (Formulation 1 and Formulation 2) were spotted on TLC plate in triplicate and run. The spot for wedelolactone in the samples were confirmed by comparing the Rf values of the spot with that of the standard. The validation parameters for the proposed method are shown in Table 1.

**Table 1. Validation parameters for wedelolactone by HPTLC.**

Parameters	Values
	Wedelolactone
Linearity range (ng/spot)	400-800
Correlation coefficient	0.9963
Regression equation (y = mx+c)	Y = 15.02 X + 2323.2
Limit of detection (ng/spot)	100
Limit of quantitation (ng/spot)	200
Precision (% RSD)	
Intra day (n=3)	0.41
Inter day (n=3)	0.26
Robustness	Robust
Specificity	Specific

#### 3.2.2 Linearity, limits of quantification and detection

Calibration plots of peak area against concentration were linear in the range 400-800 ng/spot wedelolactone. The calibration lines were represented by linear equation  $Y = 15.021 X + 2323.2$ . For this equation the correlation coefficient,  $r^2$  was 0.9963. The LOQ and LOD were calculated as 200 ng/spot and 100 ng/spot, respectively. The linearity range was found to be 400-800 ng/spot.

#### 3.2.3 Precision

The repeatability of sample application and measurement of the peak area was expressed in terms of % RSD. The % RSD was found to be less than 1.0 in all cases indicates no significant variations in the analysis of wedelolactone at the concentration of 500, 600 and 700 ng/spot.

#### 3.2.4 Robustness

The estimations were performed by varying the selected parameters within certain limits and there has been no notable alteration found on method performance and in results obtained. The developed method thus, can be considered as robust enough.

### 3.2.5 Accuracy

The accuracy was studied by the standard addition technique. Three different levels of standard were added to the previously analyzed samples, each level being repeated thrice. The percentage recovery of wedelolactone was 99.01 and 97.79 in Formulation 1 and Formulation 2 respectively, as shown in Table 2.

**Table 2. Results from recovery analysis (n=3).**

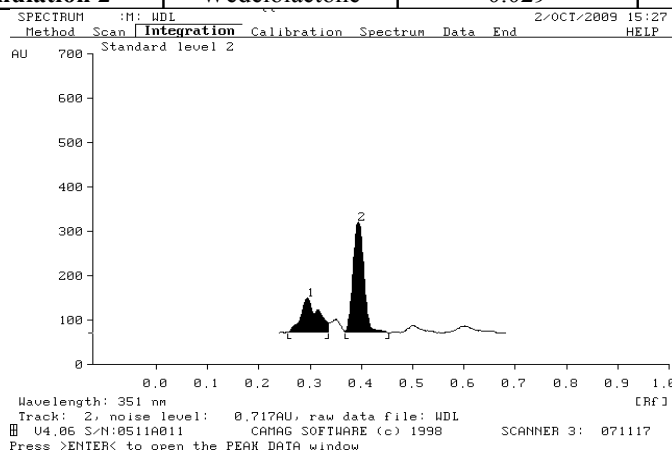
Herbal Formulations	Amount of sample taken [g] A	Amount of Wedelolactone present in (A) [µg] B	Amount of Wedelolactone added to (A) [µg] C	Total Wedelolactone taken (B+C) [µg] D	Total Wedelolactone found [µg] E	% Recovery E/Dx100 (mean)
Formulation 1	0.25	0.80	0.4	1.20	1.169	97.83
	0.25	0.80	0.6	1.40	1.376	
	0.25	0.80	0.8	1.60	1.565	
Formulation 2	0.25	0.73	0.4	1.13	1.099	96.86
	0.25	0.73	0.6	1.33	1.286	
	0.25	0.73	0.8	1.53	1.479	

### 3.2.6 Estimation of wedelolactone in herbal formulations

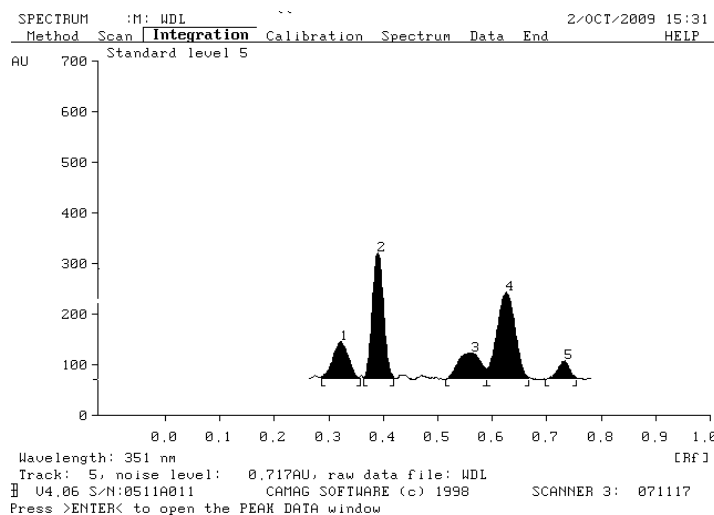
The optimized solvent system was used for the estimation of the wedelolactone from Formulation 1 and Formulation 2. The resolution was good and components were observed at different R<sub>f</sub> value, as shown in (Figure. 4, Figure. 5). The method was used to determine the wedelolactone content in Formulation 1 and Formulation 2. The results are shown in Table 3. The low RSD values are indicate the high accuracy and precision of the method.

**Table 3. Results from HPTLC analysis of Wedelolactone.**

Herbal Formulations	Component	Amount found by proposed method [% w/w]	RSD [%] (n = 3)
Formulation 1	Wedelolactone	0.032	0.444
Formulation 2	Wedelolactone	0.029	0.342



**Fig.4. HPTLC chromatogram of Formulation 1 (800 ng spot<sup>-1</sup>); peak 1–2 belong to other components present in the formulation. Peak 2 is of wedelolactone (R<sub>f</sub>: 0.39±0.03); mobile phase: toluene: ethyl acetate: acetone: formic acid (6:2:1:1, v/v/v/v)**



**Fig.5. HPTLC chromatogram of Formulation 2 (800 ng spot<sup>-1</sup>); peak 1–5 belong to other components present in the formulation. Peak 2 is of wedelolactone (Rf: 0.39±0.03); mobile phase: toluene: ethyl acetate: acetone: formic acid (6:2:1:1, v/v/v/v).**

#### 4. Conclusion

The developed HPTLC technique is a simple, precise specific, accurate and robust for the determination of wedelolactone. Statistical analysis proves that the method is reproducible and selective for the analysis of wedelolactone. Since the proposed mobile phase effectively resolves wedelolactone, the method can be used for qualitative as well as quantitative analysis of wedelolactone in herbal formulations. Further the proposed method can be extended to study the degradation of wedelolactone under different stress conditions, as per the recommendations of ICH guidelines.

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