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A Rapid Densitometric Method for Quantification of Wedelolactone in Herbal Formulations using HPTLC

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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 (Rf value of 0.39 ± 0.03). The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.9963$ 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.

Keywords: HPTLC; Wedelolactone; Herbal formulations

1. 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 [1].This plant has been well reported to exhibit hepatoprotective activity [2] and in the treatment of infective hepatitis [3]. The leaves of *Eclipta alba* showed antihyperglycemic activity [4]. A number of compounds had been isolated from the plant. Wedelolactone and de-methyl wedelolactone, complex furanocomarins have been identified as its hepatoprotective principles [5]. Literature survey revealed that HPLC [5], and UV spectrophotometry [6] methods had been reported for the estimation of wedelolactone (Fig.1) in a methanol extract. Simultaneous estimation of andrographolide and wedelolactone in herbal formulations by HPTLC method also reported [7]. Characterization of *Bhringaraj* herb by ICP-MS analysis, optical absorption, infrared and EPR spectroscopic methods also reported [8]. In the present paper, an accurate HPTLC method for quantitative determination of single wedelolactone in herbal formulations is described. The proposed method was validated in compliance with ICH guidelines [9].

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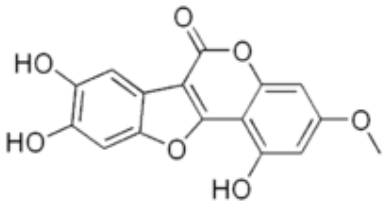

MW 314.246

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.

2.2 Standard and sample solutions

Wedelolactone (10 mg) was dissolved in 10 ml of methanol. This solution termed as stock solution of standard Wedelolactone (1 mg mL^{-1} or 1000 µg ml^{-1}). In both *Bhringaraj Ghanvati* 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.

2.3 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 $_{F-254}$ 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 ^{o}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 ^{o}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. 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 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.

2.4 Calibration curve

Standard wedelolactone solution of 100 µg mL^{-1} concentration was prepared and 4, 5, 6, 7 and 8 µL of this solution were 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. [10].

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. Figure S1 (supporting information) is showing chromatographic separation of wedelolactone at Rf 0.39±0.03. The absorption spectrum of wedelolactone is shown in (Figure S2) in supporting information. The wavelength 351 nm was used for quantification of sample.

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
Product of 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(% RSD)	Less than 1
Specificity	Specific

3.2.2 Linearity, limits of quantification and detection

Calibration plots of wedelolactone peak area against concentration were linear in the range 400-800 ng/spot. 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 introducing small changes in the mobile phase composition; the effects on the results were examined. Mobile phases having different compositions were tried and chromatograms were run. The time from spotting to chromatography and from chromatography to scanning was varied by ± 10 min. 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.

3.2.5 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	
	0.25	0.80	0.6	1.40	1.376	97.83
	0.25	0.80	0.8	1.60	1.565	
Formulation 2	0.25	0.73	0.4	1.13	1.099	
	0.25	0.73	0.6	1.33	1.286	96.86
	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 wedelolactone in Formulation 1 and 2. The resolution was good and components were observed at different Rf value, as shown in (Figure 2, Figure 3). 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.

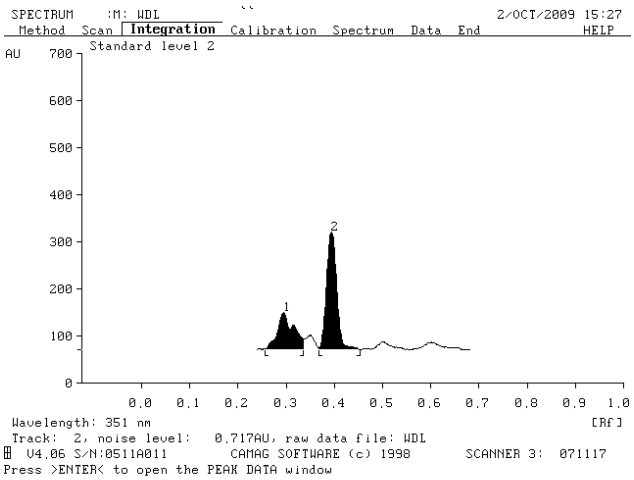

Figure 2. HPTLC chromatogram of Formulation 1 (800 ng spot−1); peak 1–2 belongs to components present in the formulation, in which, peak 2 is of wedelolactone (Rf: 0.39±0.03); mobile phase: toluene: ethyl acetate: acetone: formic acid (6:2:1:1, v/v/v/v).

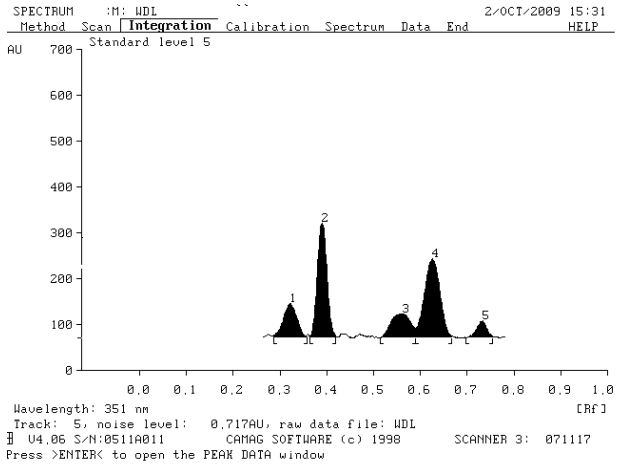

Figure 3. HPTLC chromatogram of Formulation 2 (800 ng spot−1); peak 1–5 belongs to other components present in the formulation, in which peak 2 is of wedelolactone (Rf: 0.39±0.03); mobile phase: toluene: ethyl acetate: acetone: formic acid (6:2:1:1, v/v/v/v).

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

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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