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A simple spectrofluorometric estimation of wedelolactone in methanol extract of *Eclipta alba*

Patel. M.B.* and Mishra. S.H

Herbal Drug Technology Laboratory, Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India 390 001.
Shree Leuva Patel Trust Pharmacy Mahila College, Smt. S H Gajera Shaikshanik Sankul, Chakkargadh Road, Amreli-365601.
Ph.: 02792-232321 Ext-247. Fax: 02792-232327
** For Correspondence : E-mail: mayur.pharma@gmail.com*

ABSTRACT - A simple, accurate and sensitive spectrofluorophotometric method was developed for estimation of wedelolactone, a furanocoumarin present as a major active constituent in the methanol extract of the plant *Eclipta alba*. Wedelolactone showed strong bright blue fluorescence having excitation and emission wavelength 384 and 458 nm. respectively. Linear relationship for the fluorescence intensity was obtained in the range 5-30 ng/mL. Limit of detection and limit of quantification was found to be 0.5 ng/mL and 2 ng/mL, respectively. The method was statistically validated and found suitable for estimation of wedelolactone in a methanol extract of plant *Eclipta alba.*

KEY WORDS - *Eclipta alba*, Methanol extract, Wedelolactone, Spectrofluorophotometry, Medicinal plants.

INTRODUCTION

Eclipta alba is an annual herb, has a short, flat or round stem, deep brown in color belonging to family *Asteraceae*. The plant is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation (1). There are also reports of clinical improvement in the treatment of infective hepatitis (2). *Eclipta alba* leaves showed antihyperglycemic activity (3). The roots of *Eclipta alba* were found effective in wound healing (4). *In vivo* hepatoprotective activity of alcoholic extract (5,6) and analgesic study of total alkaloids of *Eclipta alba* were also reported (7). A number of compounds had been isolated from the plant. Wedelolactone, chemically described as 7-methoxy-5, 11, 12- trihydroxy-coumestan (8), is basically a furanocoumarin, previously reported as responsible for the hepatoprotective activity (9). Literature survey revealed that HPLC (9) and UV spectrophotometry (10) methods had been reported for the estimation of wedelolactone in a methanol extract. Wedelolactone in methanolic solution shows bright blue fluorescence when observed under UV light (360 nm); therefore, it was thought to develop a more sensitive, specific, simple, precise and accurate spectrofluorometric method for the estimation of wedelolactone in methanol extract of *Eclipta alba*. Generally, fluorescence occurs because of the transition from first excited singlet state to ground state by emission of light (11). Wedelolactone consist of heterocyclic fused ring, which is responsible for fluorescent behavior.

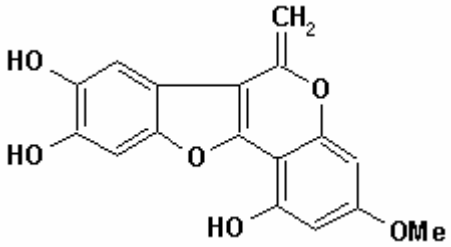

Figure: 1 Chemical structure of Wedelolactone

MATERIALS AND METHOD

A Shimadzu spectrofluorophotometer (Tokyo, Japan, Model RF-540 with DR-3 data recorder), equipped with a 1 cm fluorescence free quartz cell was employed for all spectral and fluorescence measurements. Instrumental parameters were as follows:

Scan speed: Medium, Sensitivity: High, Abscissa scale: 2, Ordinate scale: 3

Pure authentic sample of wedelolactone obtained as gift from M/s Laila impex, Vijayavada. Silicagel G used for Preparative TLC was procured from Loba Chemie (Mumbai, India). Methanol, toluene, acetone, and formic acid used were of AR grade and were procured from Qualigens (Mumbai, India). Whatman filter paper no. 42 [whatman, London] was used to filter the solution. Fresh herb of *Eclipta alba* was collected from

the local area of Vadodara in October, 2004. Botanical authentication was performed at the Botany Department of The M S University of Baroda, Vadodara, and specimen preserved in the department. Collected plant material was dried under shade; aerial parts were separated from the herb and grounded in to # 10 powder.

Preparation of standard solution for calibration curve

Standard solution (100 µg/mL) was prepared by dissolving 5 mg wedelolactone in 50 mL methanol. Standard stock solution of the concentration 5 µg/mL was prepared by diluting 2.5 mL of the above solution to 50 mL with methanol. Appropriate dilutions were made in methanol to produce working stock solutions of 50, 100, 150, 200, 250, and 300 ng/mL. The standard curve of wedelolactone was plotted by measuring fluorescence intensity of solutions containing 5, 10, 15, 20, 25 and 30 ng/mL concentration.

Preparation of sample solution

Exactly weighed 50 grams powdered drug was extracted with methanol in Soxhlet apparatus at 70 ^{0}C for 6 hrs. Methanol extract was concentrated to about 25 mL and then dried *in vacuo* oven at 50^{0}C for 3 h (23.34 % w/w) A solution of extract (1 mg/mL) was prepared in methanol. With the use of marked capillary, 100 µL of the resultant solution was applied on the chromatographic plate as a band along with a reference spot of standard wedelolactone. Preparative TLC was run using Silicagel G as a stationary phase and a mobile phase (11) consisting of toluene:acetone:formic acid (11:6:1,by volume). Visualization of wedelolactone was performed under U.V. Chamber, having R_f value 0.56 and distinct bright blue fluorescence. The band of wedelolactone was scrapped off using sharp blade, extracted with methanol (3 X 10 mL) and filtered through Whatman filter paper no.42, the residue on the filter paper was washed with methanol and final volume of the solution was made up to 50 mL. It was considered as a sample stock solution. 5 mL of this solution was adjusted to 10 mL and the fluorescence intensity was measured.

Estimation procedure

Standard solution of wedelolactone was scanned in the range of 300-600 nm for determination of excitation wavelength and it was found to be 384 nm. Same solution was scanned for emission wavelength in the range of 370-600 nm taking 384 nm as an excitation wavelength and it was found to be 458 nm.

RESULTS AND DISCUSSION

The fluorescence intensity of wedelolactone has linear relationship in the concentration range of 5-30 ng/mL. Stability study proved that the fluorescent characteristic of the solution was stable up to 3 h at room temperature (28 ± 2 ^{0}C). After that, fluorescence intensity diminished gradually. Other parameters like excitation wavelength, emission wavelength, regression equation, coefficient of determination (r^2), correlation coefficient (r), limit of detection (LOD), limit of quantification (LOQ) are listed in Table 1. Recovery study was carried out by adding known amount of pure wedelolactone to preanalysed crude drug and again analysed to estimate wedelolactone content. The concentration of wedelolactone in all samples under investigation was calculated with the help of the standard curve. The developed method was found to be specific, accurate, simple, precise, and reproducible and hence can be used for routine analysis of methanol extract of *Eclipta alba* for wedelolactone content.

It has always been a challenging task to estimate marker compound from the extract, as the separation of chemical moieties becomes the prior condition to perform HPTLC quantification. At present, wedelolactone in the extracts was only estimated using the sophisticated instruments because the reported UV spectrophotometric method lacks specificity. This has, therefore, necessitated developing a new specroflurometric method using the separated compound of the interest by preparative TLC. The method was found to be highly selective without any interference. The LOD and LOQ were found to be within the detectable and quantifiable limits. The validation parameters reported, assures the competitive performance of the newly developed method for estimating the marker compound from the complex matrix. The present studies were performed to evolve a new analytical method, which can help in ongoing research on standardization of various herbal and traditional formulations in practice. The developed method was found to be specific, accurate, simple, precise, and reproducible and hence can be used for routine analysis of methanol extract of *Eclipta alba* for wedelolactone content.

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Table No. 1- Optical Characteristics and Analytical Data

Parameters	Results
Excitation wave length	384 nm
Emission wave length	458 nm
Linearity range (ng/mL)	5-30
Regression equation (Y^a)	Y = 2.7391 X + 4.0727
Slope (b)	2.7391
Intercept (a)	4.0727
Coefficient of determination (r^2)	0.9954
Correlation coefficient (r)	0.9977
Limit of Detection (ng/mL)	0.5
Limit of Quantification (ng/mL)	2
Accuracy (% w/v)	> 98 %

Table No.2 - Data of Calibration Curve for Wedelolactone

Concentration of Wedelolactone (ng/mL)	# Relative Fluorescence Intensity	% RSD
5	20.37	0.55
10	29.94	1.36
15	43.21	0.63
20	58.02	0.87
25	73.15	0.58
30	87.35	0.43

 # Mean of five determinations

Table No. 3 - Data of Sample Analysis and Recovery Study

Sample	% w/w of Wedelolactone	Amount of wedelolactone In pre analyzed sample (ng/mL)	Amount of Std. wedelolactone added.		Total amount of wedelolactone in solution (ng/mL)	Actual amount of wedelolactone found during recovery study* (ng/mL)	% Recovery
			% added	ng added			
Methanol Extract	5.373	12.71	0	0	12.71	12.53 ± 0.95	98.58 %
		12.71	80	10	22.71	22.41 ± 1.94	98.68 %
		12.71	100	13	25.71	25.82 ± 1.87	100.43 %
		12.71	120	16	28.71	28.47 ± 1.92	99.16 %

* *Mean of five determinations.*

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