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Development of a simple and sensitive spectrophotometric method for the simultaneous determination of asiaticoside and wedelolactone in a polyherbal formulation.

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ABSTRACT - A simple, sensitive spectroscopic method was developed for the simultaneous determination of asiaticoside and wedelolactone in mixture of *Centella asiatica* extract and *Eclipta alba* extract as well as in a polyherbal formulation containing these extracts. Asiaticoside has an absorption maxima at 278nm, the molecule obeys Beer's law in the concentration range 10-100 µg/mL, while wedelolactone has the absorption maxima at 351nm and the linearity range is 1-10 µg/mL. The method for simultaneous estimation was based on the property of additive values of absorbance. The proposed method is reproducible and statistically validated. The average % recovery for asiaticoside is 98.63 % and for wedelolactone is 98.41 %. The developed method is an effective approach for simultaneous quantitative determination, which could be used for quality control of polyherbal preparations containing extracts of *Centella asiatica* and *Eclipta alba*.

KEY WORDS: Asiaticoside, Polyherbal, Simultaneous Determination, Wedelolactone.

INRODUCTION

Centella asiatica (Linn) Urban; syn. *Hydrocotyl asiatica* Linn known as Indian pennywort is a prostate, perennial, faintly aromatic herb found wild throughout India (1,2). The plant enjoys considerable reputation in Indian system of medicine as diuretic, alternative and tonic (3). An infusion of the plant is used in India in the treatment of leprosy (4, 5). The plant contains different components such as essential oils, fatty acids, phytosterols, polyacetylenes and saponins, out of which the α-amyrin derivative, asiaticoside is one of the most active compounds which can serve as a marker (3, 4) and is used for its standardization. A few methods such as gravimetric (6) and column chromatography (7) have been suggested for the quantitative estimation of asiaticoside which are not very precise, sensitive and require multiple step extraction and purification. Literature reveals that HPLC (8, 9, 10) and HPTLC (11) methods are also available.

Eclipta alba is another well known herb which has significant medicinal value in hepatic and splenic disorders and various chronic skin diseases. It contains mainly coumestan derivatives, wedelolactone and demethylwedelolactone, thiophene derivatives, triterpenoids, flavonoids, alkaloids and polypeptides (12).HPLC (12), UV Spectrophotometric (13) and Spectrofluorometric (14) methods are reported for the estimation of wedelolactone. It is found in many of the traditional as well as the modern system of medicine in combination with *Centella asiatica* for a number of indications like skin disorders and to stimulate hair growth. A simple, precise and accurate method for the simultaneous determination of these components in a mixture present in the formulations has not been reported yet. An attempt, therefore, has been made in the present study to develop a new method for simultaneous estimation of wedelolactone and asiaticoside in polyherbal formulations. This method could be used in the routine estimation of asiaticoside and wedelolactone in the dosage forms.

MATERIALS AND METHODS

Instruments and Reagents

Shimadzu 1601 UV, Japan recording spectrophotometer with 10 mm matched quartz cells was employed for this work. Authentic Samples of asiaticoside as well as wedelolactone were obtained as a gift samples from M/s Laila Impex, Vijaywada. Methanol (analytical grade reagent) was obtained from Qualigens (Mumbai, India) was used in the study.

Preparation of standard solutions for calibration

Standard stock solutions of asiaticoside and wedelolactone were prepared in methanol separately and suitable aliquots were taken and diluted to 10 mL with methanol. Both the standard solutions were

scanned in UV range of 400 nm to 200 nm and their individual spectrums were superimposed to obtain the best suitable wavelengths in order to develop simultaneous estimation methods. The λ max for asiaticoside was found to be 278 nm and that of wedelolactone was 351 nm. Superimposed UV spectra of wedelolactone and asiaticoside standards is shown in Fig. 3

Fig 1. Chemical structure of wedelolactone

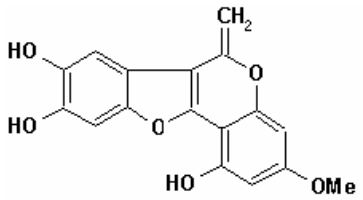
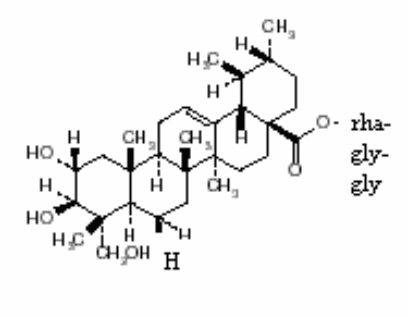


Fig 2. Chemical structure of asiaticoside



Applicability of Beer's law at the above wavelengths

The linearity range was found to be in the concentration series from 10-100µg/mL for asiaticoside and 1-10 µg/mL for wedelolactone. The linear regression equation was y = 0.0072 x – 0.045 with a slope (a) was 0.0072, intercept (b) was -0.045 and coefficient of determination (r^2) was 0.9992.

Preparation of Samples

Mixture of Centella asiatica and Eclipta alba extract

Centella asiatica and *Eclipta alba* were collected from the local area of Baroda and the botanical authentication was done at the botany department of M.S. University of Baroda. The voucher specimens are maintained in the herbarium at Pharmacy Department, The M.S. University of Baroda. The collected plant material was dried in shade and size reduced to 12# powder.

Solutions of methanol extract of *Centella asiatica* and *Eclipta alba* were prepared in methanol, concentration 4 mg/mL each. 2.5 mL of each solution was mixed and a number of aliquots from this mixture in the concentration ranging from 0.2, 0.4, 0.6, 0.8, 1.0 mL were taken in separate volumetric flask and diluted. These samples were then scanned over the range of 400 nm to 200 nm in the multicomponent mode.

Formulation

The average weight of the capsules was determined by weighing 20 capsules and the hard gelatin capsules were opened and the contents were finely powdered. 50 mg of the powder was transferred in to a volumetric flask containing small amount of methanol and sonicated for 10 minutes followed by dilution up to 100 mL with methanol. The resultant solution was centrifuged and the supernatant was taken for estimation.

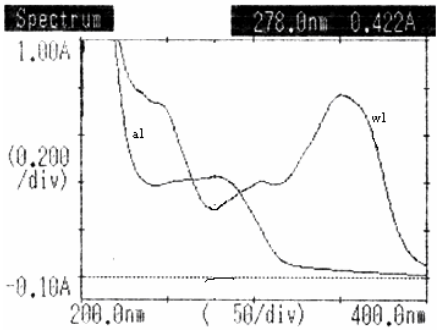
Estimation procedure

Based on the property of additive values of absorbance (15, 16,).

Standard calibration curves of asiaticoside and wedelolactone were plotted at 278 nm and 351nm. Wedelolactone showed an absorbance peak at 351 nm, where asiaticoside showed least absorbance. Hence, concentration of wedelolactone in the sample solution was obtained from the absorbance of sample solution at 351 nm and calibration curve plotted at 351 nm.

Fig 3. Superimposed UV Spectra in the range of 400-200 nm.

a1- Spectra of Asiaticoside. w1- Spectra of Wedelolactone



Asiaticoside showed an absorbance peak at 278 nm. The concentration of asiaticoside in the sample solution was obtained from the absorbance contribution of asiaticoside to the sample solution at 278 nm as follows:

$$A_1 = A - A_2$$

A_1 = Absorbance contribution of asiaticoside at 278 nm.

A = Absorbance contribution of the sample solution at 278 nm.

A_2 = Absorbance contribution of wedelolactone to the sample solution at 278 nm.

The absorbance contribution of wedelolactone to the sample solution (A_2) was obtained from the concentration of wedelolactone in the sample solution and its calibration curve plotted at 278 nm.

Table 1. Results of the Analysis for the Quantitative Determination of Asiaticoside and Wedelolactone by the Proposed Method

S.No.	Sample Mixture of extracts-Volume of stock solution in the sample	% of Asiaticoside[#]	% of Wedelolactone[#]	% RSD
1	0.2	17.16	4.96	0.89
2	0.4	18.35	5.31	0.96
3	0.6	17.56	5.01	0.76
4	0.8	16.55	5.15	1.10
5	1.0	17.03	5.06	0.45
6	Formulation	16.23	5.29	0.65

[#]*Mean of five determinations*

Table 2. Data of recovery study for Asiaticoside in formulation

S.No.	Amount of Asiaticoside In pre analyzed sample %	Amount of Asiaticoside added		Total amount of Asiaticoside in solution (µg/ml)	Actual amount of Asiaticoside found during recovery study (µg/ml)	% Recovery
		%	µg			
1	16.23	0	0	16.23	16.095 ±0.67	99.17
2	16.23	80	12.98	29.21	29.027±0.93	99.36
3	16.23	90	14.60	30.84	30.105± 1.37	97.63
4	16.23	100	16.23	32.46	31.99± 1.62	98.55
5	16.23	110	17.85	34.08	33.715± 0.93	98.92
6	16.23	120	19.47	35.71	35.049± 0.59	98.16
					Average	**98.63**

Table 3. Data of recovery study for Wedelolactone in formulation

Amount of Wedelolactone In preanalyzed sample%	Amount of Wedelolactone Added		Total amount of Wedelolactone in solution (µg/ml)	Actual amount of Wedelolactone found during recovery study (µg/ml	% Recovery
	%	µg			
5.29	0	0	5.29	5.11± 0.94	96.59
5.29	80	4.232	9.522	9.49± 1.12	99.66
5.29	90	4.761	10.051	9.99± 1.23	99.39
5.29	100	5.29	10.58	10.38± 0.87	98.10
5.29	110	5.819	11.109	11.09± 1.34	99.82
5.29	120	6.348	11.638	11.28± 0.45	96.92
				Average	**98.41**

Recovery study

A varying known amount of asiaticoside were added to about 1 mL of the test samples in which the contents of asiaticoside had been estimated previously by proposed method. The contents of asiaticoside were quantified using proposed method and percentage recovery was calculated. Similar method was followed for wedelolactone. The results are provided in Table 2 and 3.

Sensitivity (17)

For limit of detection first the absorbance "*p*" was calculated by following equation

$$p = b+3SD$$

Where *b* is the average of five replicate readings of blank and SD is standard deviation of five replicates. The value of limit of detection was calculated by comparing the absorbance "*p*" with the absorbance of known concentration of sample. The limit of quantification (LOQ) was determined by taking the ratio of the standard deviation of the blank with respect to water and the slope of the calibration curve multiplied by the factor 10.

$$LOQ = \frac{10\,\sigma}{S}$$

$$LOD = \frac{3.3\sigma}{S}$$

Where:

σ = Standard deviation of the response

S = Slope of the calibration curve.

This means that LOQ is approximately 3.3 times greater than LOD.

RESULTS AND DISCUSSION

The proposed method was developed using spectrophotometric technique and found to be accurate, simple and convenient for simultaneous analysis of asiaticoside and wedelolactone in pharmaceutical formulation. The modalities adopted in experimentation were successfully validated as per standard analytical procedures.

Beer's law is obeyed in the concentration range of 1 to 10 µg/mL of wedelolactone and 10-100 µg/mL of asiaticoside with the coefficient of determination (r^2) was 0.9992. The accuracy of the method was proved by two ways, first by direct determination of asiaticoside and wedelolactone in extract, second by determination commercially available formulation.

The analytical results obtained for the simultaneous determination of asiaticoside and wedelolactone in the extract and formulation are listed in Table-1.

The LOD and LOQ were found to be 0.1456 µg/mL and 4.394 µg/mL respectively. LOD is well below the lower limit of the Beer's law range. The average % recovery for asiaticoside was 98.63 % and for wedelolactone was 98.41 %, showing that the method does not suffer from any interference due to other constituents and common excipients. Rigorous analysis of the results shows that the presence of other constituents in the formulation did not interfere with the final determination of active components.

The WHO has emphasized the need to develop the quality control parameters for herbal products and in this connection the present method of estimation of asiaticoside and wedelolactone content of the formulation is very useful. It is a simple, precise and accurate method suitable for the routine analysis in pharmaceutical preparations.

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