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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF HIBIFOLIN ISOLATED FROM THE ETHANOLIC EXTRACT OF *MELOCHIA CORCHORIFOLIA* LEAVES

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ABSTRACT

Simple, precise and accurate zero orders derivative spectroscopic method has been developed and validated for the estimation of Hibifolin. The drug shows maximum absorption (λ_{max}) at 664nm in Methanol solution and obeys Beer's law in the concentration range of 50-250µg/ml. The linearity study carried and regression coefficient was found to be 0.9997 and it has showed good linearity, precision in this concentration range. The LOD and LOQ were found to be 2.6332 and 7.9014µg/ml. The % relative standard deviations were found less than 2. The method has been validated according to ICH guidelines for linearity, precision, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for reliable quantification of Hibifolin.

Keywords *– Hibifolin, Zero order derivative spectroscopy, Validation, Isolated compound.*

1. INTRODUCTION

Melochia corchorifolia is also known as Chocolate weed, is a weed plant this is typically seen in wastelands and frequently observed in open areas, such as highways. [1] *Melochia corchorifolia* plant extracts shows Hepatoprotective, Antioxidant, [2] Anthelmintic, [3] Anticancer, [4] Diuretic, Antiurolithiatic [5] and CNS Stimulant activity. [6] The chemical constituents present in the *Melochia corchorifolia* leaves extract Shows, Alkaloids: The alkaloids are franganine, frangufoline adouetine-y' and melofoline (cyclopeptide alkaloids), melochironine (a pseudo-oxindole alkaloid) and 6- methoxy-3-propenyl-2-pyridine (pyridine alkaloid). Pyridine derivatives: pyridoxine, 4-methoxypyridine, nicotinic acid. Flavonol glycosides: Hibifolin, triflin and melofoline. Triterpenes: friedlin, friedelinol and β-amyrin. Flavonoids: vitexin and robunin. Aliphatic compounds: Ethyl stearate, tetratriacontanol, nonacosylnon-4-enoate, 24-ethyl-2- metyltritetracont-1-ene-3, 23-diol and 27-methyloctacosane-1, 3-diol. And β-D-sitosterol, stearate, D-glucoside, cyclopeptide alkaloids, a new pseudooxindole alkaloid, melochironine active compounds have been isolated from *M.corchorifolia.* [7- 11]

Hibifolin is a Flavonol glycoside isolated from the ethanolic extract of *Melochia corchorifolia* leaves. Molecular weight and Chemical formula of Hibifolin is 493.36 g/mol and $C_{21}H_{18}O_{14}$ respectively.

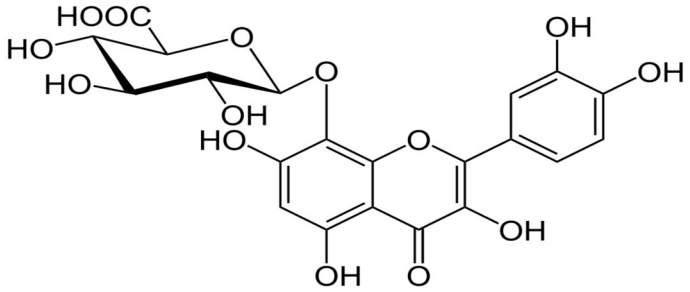

Fig. 1. Structure of Hibifolin

The aim of present work is to develop and validate a novel, rapid, simple, precise, and specific Zero order derivative UV Spectrophotometric method for estimation of Hibifolin isolated from the ethanolic extract of *Melochia corchorifolia* leaves.

2. MATERIALS AND METHOD [12, 13]

2.1 Instrument

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken on analytical balance.

2.2 Chemicals

Hibifolin isolated from the ethanolic extract of *Melochia corchorifolia* leaves.

2.3 Solvent

Methanol.

2.4 Selection of analytical wavelength

Appropriate dilutions of Hibifolin isolated compound were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 400-800nm. The absorption spectra obtained and show maximum absorbance at 664nm which was selected as the wavelength for detection (Fig-2).

2.5 Preparation of standard stock solution

100 mg of Hibifolin isolated compound was weighed accurately and transferred in to 100ml volumetric flask and dilute in methanol up to mark. from this solution pipette out 0.5, 1.0, 1.5, 2.0 and 2.5ml into 10ml individual volumetric flask and dilute in methanol up to mark, this gives 50, 100, 150, 200 and 250µg/ml concentration.

2.6 Method validation

The method was validated according to ICH guidelines.

3. Results and Discussion

Method: Zero order derivative spectroscopy.

3.1 Linearity

The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 50-250µg/ml was measured at 664nm and absorbance values are shown in table-1. The calibration curve was prepared by plotting graph against the concentration and absorbance and the graph shown in Fig-3. Statistical parameter like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined. (Table-2).

3.2 Precision

The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the same sample. Precision was determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for three times in a same day. Inter-day precision was determined by analysing the same concentration daily for three days. (Table-3).

3.3 Ruggedness

The ruggedness is defined as the reproducibility of results when the method is performed under the variation in conditions. This includes different analyst, laboratories, instruments, temperature etc. Ruggedness was determined between different analysts, the value of %RSD was found to be less than 2. (Table-5).

3.4 Limit of detection and Limit of Quantitation

The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated using formula.

LOD = 3.3(SD)/S and LOQ = 3(LOD)

LOD and LOQ value of Hibifolin were found to be 0.0328 and 0.0984µg/ml.

Table 1: Results of calibration curve at 664 nm by zero order spectroscopy

SL. No	Concentration in µg/ml	Absorbance ±Standard deviation*
1	0	0
2	50	0.082 ±0.00518
3	100	0.162 ±0.00859
4	150	0.239±0.00680
5	200	0.318 ±0.00972
6	250	0.405 ±0.01648

*Average of six determinations.

Table 2: Regression parameter for Hibifolin by zero order spectroscopy

Regression parameter	Results
Range(µg/ml)	50-250
λ_{max}(nm)	664
Regression Equation	Y= 0.0016x+0.0003
Slope(b)	0.0016
Intercept(a)	0.0003
Correlation coefficient(r^2)	0.9997
Sandell's equation	0.638
Limit of detection(µg/ml)	2.6338
Limit of quantitation(µg/ml)	7.9014

Table 3: Determination of precision results for Hibifolin at 664 nm by zero order spectroscopy

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
5	0.083±0.0017	1.2	0.083±0.00124	1.44
10	0.164±0.00286	1.21	0.166±0.00124	0.602
15	0.238±0.00163	0.42	0.241±0.00249	0.995
20	0.320±0.00286	0.87	0.322±0.0033	1.024
25	0.406±0.00411	1	0.412±0.00244	0.582

**Average of six determinations, **percentage relative standard deviation.*

Table 4: Determination of Ruggedness results for Hibifolin at 664nm by Zero order spectroscopy

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.237	0.242
±Standard deviation*	0.00286	0.00244
%RSD**	0.843	0.991

**Average of three determinations, **percentage relative standard deviation.*

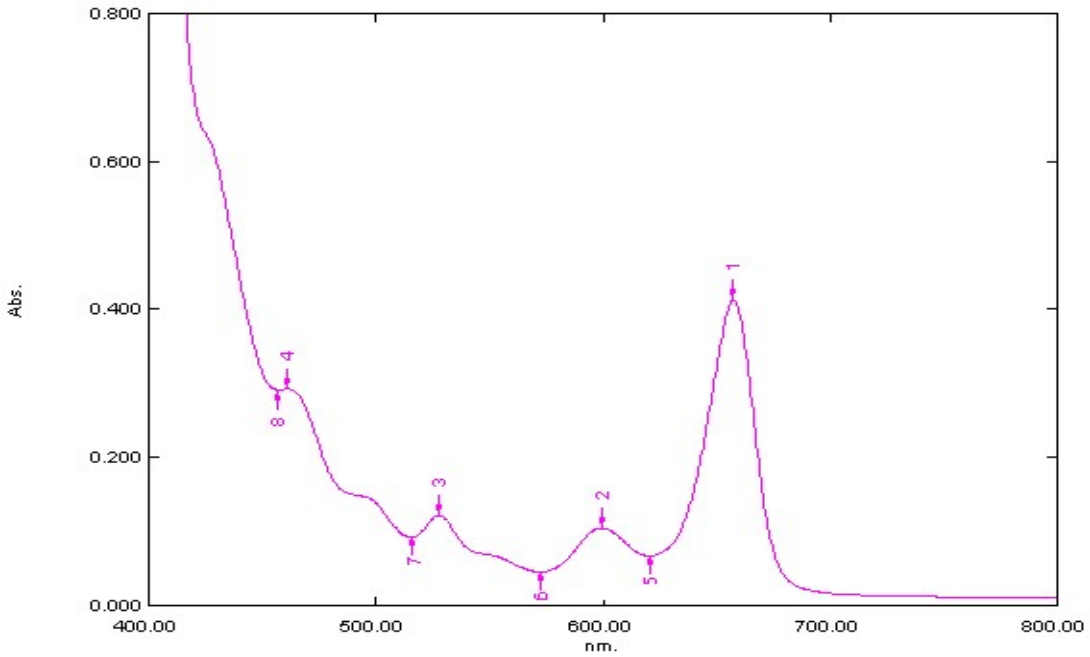

Fig.2: Zero order spectrum of Hibifolin at 664nm

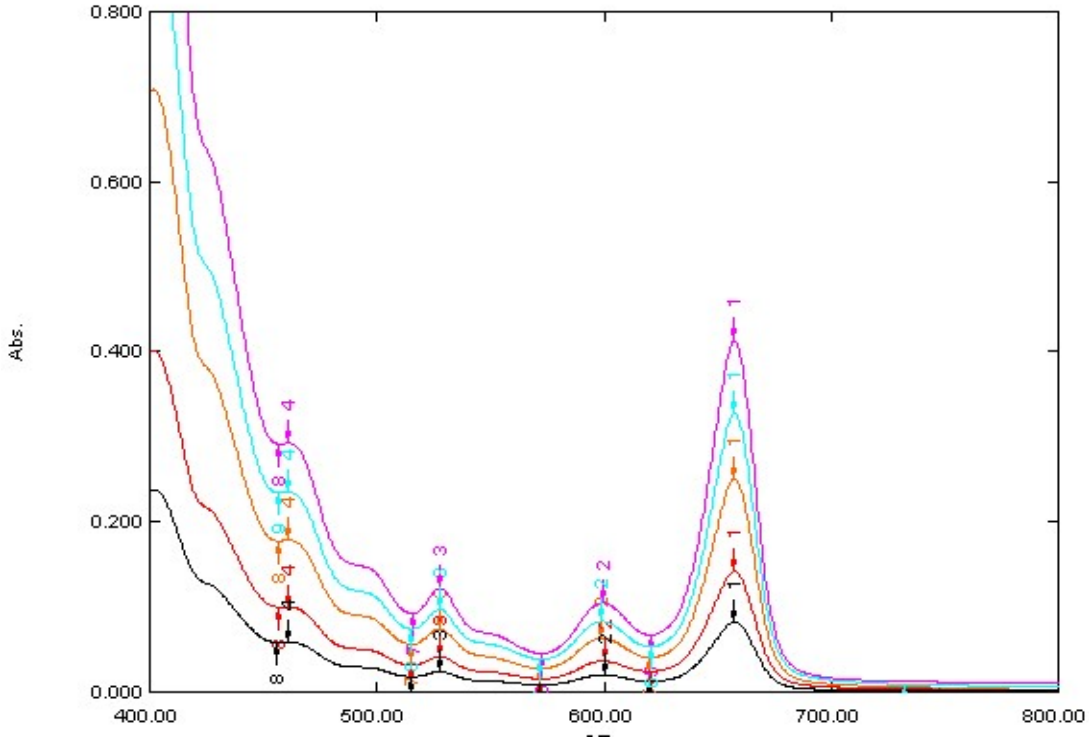

Fig.3: Zero order overlain spectra of Hibifolin showing absorbance at 664nm

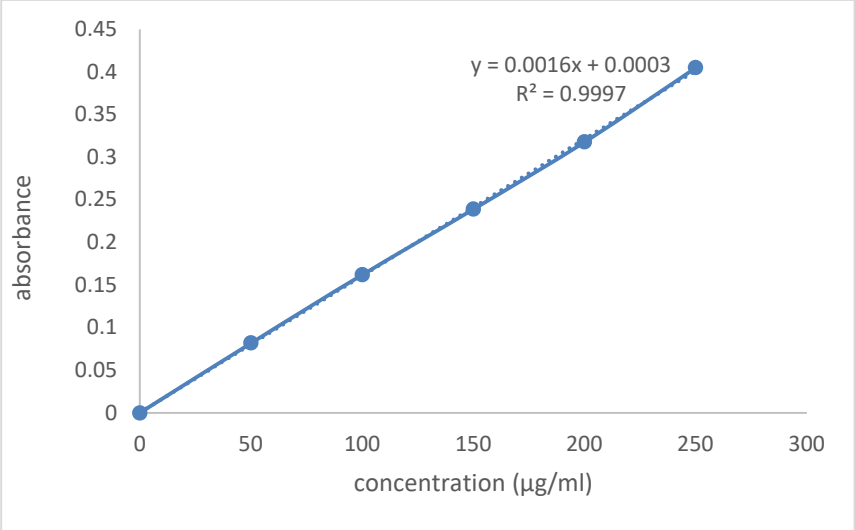

Fig.4: Calibration curve of Hibifolin by zero order spectroscopy

4. CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, specific, economical and sensitive and can be used for routine analysis of Hibifolin.

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