

Spectrophotometric Estimation of Ferulic Acid from *Ferula asafoetida* by Folin - Ciocalteu's Reagent

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ABSTRACT

*A simple, sensitive and reproducible spectrophotometric method has been developed by means of Folin-ciocalteu's reagent in 15 % sodium carbonate for quantitative estimation of ferulic acid from *Ferula asafoetida*. The blue coloured chromogen thus formed after the reaction was measured at wavelength of maximum absorption 718 nm for ferulic acid against the blank reagent. The chromogen obeyed linearity over the range of 1µg/ml - 8µg/ml. The method was further validated as per ICH guidelines by studying various parameters such as linearity, precision, accuracy, limit of detection and limit of quantification.*

Keywords: Ferulic acid, Folin-ciocalteu reagent, *Ferula asafoetida*, Spectrophotometry.

INTRODUCTION

Ferula asafoetida syn *Ferula foetida* Regel belongs to family Umbelliferae is perennial plant which grows to about 2 m by 1.5 m and requires dry or moist soil. The dried latex which is known as asafoetida is obtained by making deep incisions in the root and rhizomes are preferred for the medicinal as well as culinary use [1]. The constituents present in asafoetida are resin (40 to 65 %), gum (20 to 25 %) and volatile oil (4 to 20 %). The resin portion comprises chiefly of a sesquiterpene coumarin which is assaresinotannol in free or in combined form alongwith ferulic acid. Ferulic acid is a phenolic acid which is present in asafoetida and furnishes with a number of activities (2)(3). Literature survey reveals that asafoetida furnishes with a number of activities such as antispasmodic, antifungal, antioxidant, anti-diabetic, anti-microbial, antiulcer, antihaemolytic, chemopreventive, and antiviral [4-11]. Hence an attempt has been made to develop a simple, sensitive, repeatable and cost effective VIS-Spectrophotometric method for the determination and quantification of ferulic acid from *Ferula asafoetida* using Folin-Ciocalteu reagent in presence of 15 % sodium carbonate.

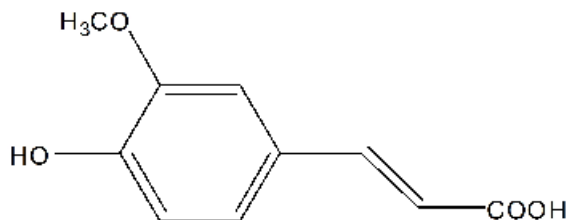


Fig 1. Ferulic acid, 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid

MATERIALS AND METHODS

Instrument

A Jasco model V-630 double beam UV/VIS spectrophotometer operated by SPECTRAMANAGER software with 1.0 cm matching quartz cells were used for absorbance measurement in the visible regions.

Chemicals

AR grade chemicals such as methanol, Folin – Ciocalteu reagent and sodium carbonate were obtained from S. D. Fine chemicals. Double distilled water was obtained after purification from ELGA Ferulic acid of 98% purity was purchased from P. C. Chem (Mumbai, India).

Plant material

The dried oleo-gum-resin of asafetida was obtained from Yucca Enterprises Mumbai. The oleo-gum-resin was powdered and kept in an air tight container.

Reaction Mechanism of Folin–Ciocalteu reagent

The Folin–Ciocalteu reagent which is a mixture of tungstates and molybdates works on the mechanism of oxidation–reduction reaction. The method strongly relies on the reduction of the mixture heteropolyphosphotungstates–molybdates by the phenolic compound which results in the formation of blue coloured chromogen. The phenolic compounds react with Folin–Ciocalteu reagent only under basic conditions adjusted by sodium carbonate solution. Under Basic conditions it has been observed that the phenolic compound undergoes dissociation to form a phenolate anion which reduces the Folin–Ciocalteu reagent i.e. the mixture of tungstates and molybdates rendering a blue coloured solution. The colour intensity of the formed blue chromogen can be measured by the absorbance readings using a spectrophotometer [14-17].

Preparation of standard stock solution of ferulic acid

A stock solution of ferulic acid (1 mg/ml) was prepared by dissolving 10 mg of accurately weighed ferulic acid in methanol and making up the volume to 10 ml with methanol in a 10 ml volumetric flask. From this 1 ml was pipette out and transferred to a 10 ml volumetric flask and the volume was made upto 10 ml by adding required volume of double distilled water to get a concentration of 100 $\mu\text{g/ml}$ which was further used for preparing solutions for calibration curve.

Preparation of calibration curve of ferulic acid

From the aforesaid stock solution of 100 $\mu\text{g/ml}$ aliquots of 0.1 ml to 0.8 ml were transferred to series of 10 ml volumetric flask. To each flask 2 ml of 15 % sodium carbonate solution and 0.5 ml of Folin–Ciocalteu reagent diluted with double distilled water in the ratio 1:2 was added and the required volume was made up by adding double distilled water in order to get a solution in the concentration range of 1 $\mu\text{g/ml}$ - 8 $\mu\text{g/ml}$ of ferulic acid. The mixture gave maximum absorption at wavelength of 718 nm when the spectra of the mixture were determined against a blank solution. The absorbances of all the solutions were measured at 718 nm against a blank solution and the calibration curve were plotted by considering the absorbance readings against their corresponding concentration by linear least square regression analysis.

Preparation of Methanolic Extract of *Ferula asafoetida*

Accurately weighed 25 g of asafetida powder was extracted with 100 ml methanol and it was subjected for filtration. The filtrate was evaporated to get a brown extract, which was used for further analysis.

Preparation of sample solution

50 mg of the methanolic extract was accurately weighed and transferred in a 25 ml volumetric flask containing 20 % methanol and the volume was made upto 25 ml by the adding required volume of the aforesaid solvents. The solution was then filtered through a Whatman filter paper No. 41. Aliquots of 1ml of the extract solution was transferred to a 10 ml volumetric flask and to the volumetric flask was added 0.5 ml of Folin – Ciocalteu reagent diluted with double distilled water in the ratio 1:2 and 2 ml of 15 % sodium carbonate solution and volume was adjusted by adding double distilled water upto the 10 ml mark. The absorbance was measure at 718 nm against the blank solution.

Validation of the proposed method**Linearity**

The linearity was determined by constructing the calibration curve and evaluating it by linear least square regression analysis.

Accuracy as Recovery

The accuracy of the method was determined by calculating recoveries of ferulic acid by the method of standard addition. To the prequantified samples about 50 %, 100% and 150% of standard ferulic acid were added. The amount was determined by measuring at 718 nm. The recovery was verified by estimating the markers in triplicate samples at each specified concentration levels.

Limits of Detection and Quantification

The limit of detection (LOD) of an analytical procedure is the lowest amount of analyte in a sample which can be detected but cannot be quantitated as an exact value as such. LOD was calculated using the formula $3.3 \times (\text{standard deviation of y-intercept}) / \text{slope of the calibration curve}$. The limit of quantification (LOQ) of an analytical procedure can be defined as the lowest amount of an analyte in a sample which can be quantitatively determined. LOQ can be calculated by using the formula $10 \times (\text{standard deviation of y-intercept}) / \text{slope of calibration curve}$.

Precision

The intraday precision was determined by estimating the corresponding response on the same day whereas the interday precision was determined by estimating the corresponding response on different days over a period of one week.

RESULTS AND DISCUSSION**Determination of UV max**

The mixture of ferulic acid alongwith the Folin – Ciocalteu reagent in an alkaline medium yielded maximum absorbance at 718 nm whereas the spectra of ferulic acid shows a UV max at 319 nm.

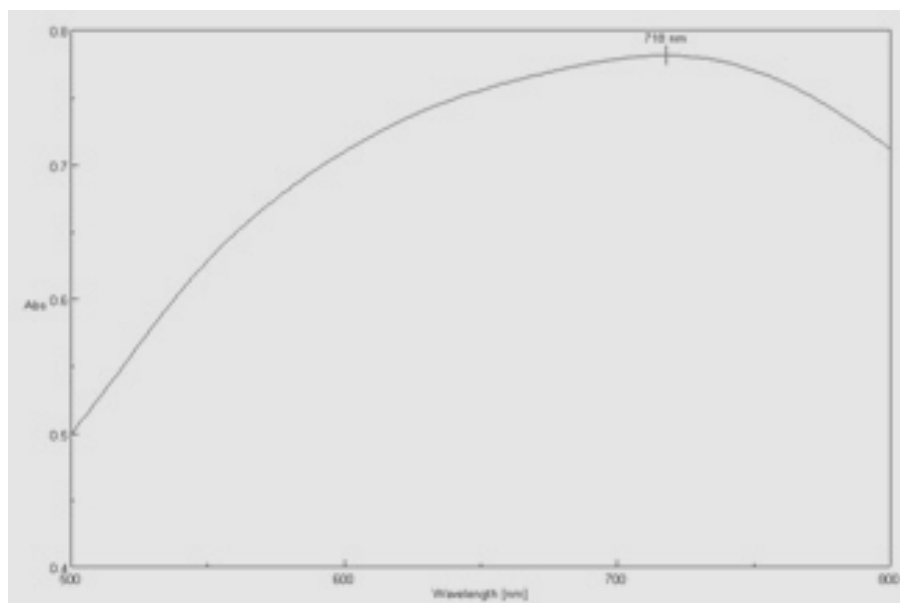


Fig .2 Spectra of mixture of Ferulic acid and Folin – Ciocalteu reagent in an alkaline medium

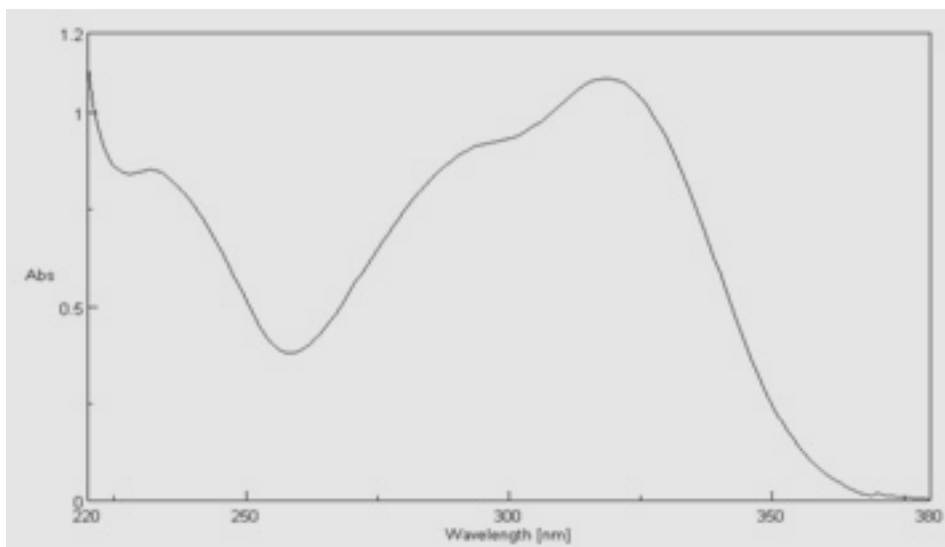


Fig. 3 Spectra of Ferulic Acid

Estimation of ferulic acid

The amount was calculated as total phenolic acid content equivalent to ferulic acid and was found to be 2.518 %.

Linearity

A linear relationship was obtained when a graph was plotted for concentration v/s absorbance in the concentration range of 1µg/ml - 8µg/ml with a co-relation coefficient value $r^2 = 0.997$ and the linear regression equation was $y=0.068x + 0.025$ (Table No.1).

Table. 1 (Regression Analysis Data)

Regression Equation	$y = 0.068x + 0.025$
Range	1µg/ml - 8µg/ml
Co-relation coefficient r^2	0.997
Slope m	0.068
y-intercept	0.025

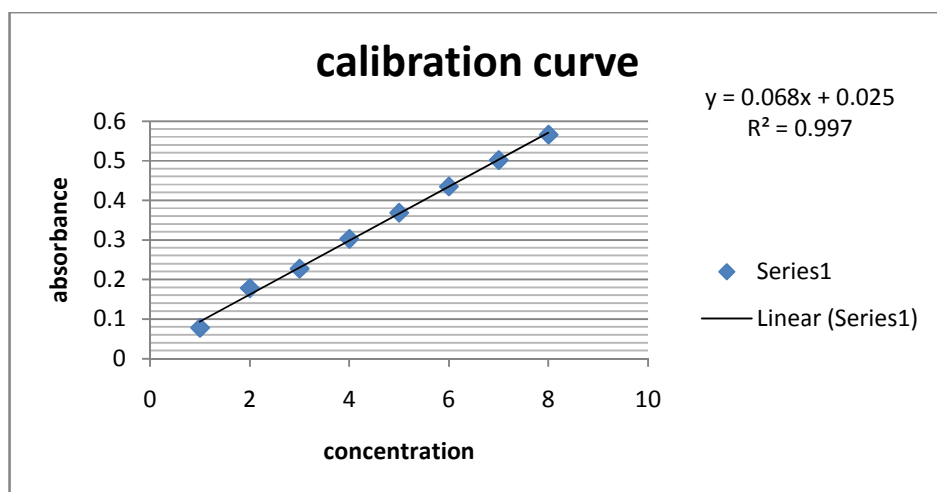


Fig. 3 Calibration Curve for Ferulic acid

Accuracy as Recovery

The proposed method yielded an average recovery of 98.07 % when the standard ferulic acid was spiked in the sample solution and analyzed by the proposed method (Table No. 2).

Table. 2 Accuracy as Recovery

Amount of Marker added %	Amount of Marker added μg	Amount of Marker found μg	Recovery %	Average Recovery %
50 %	2.518 μg	2.5087 μg	99.59 %	98.07 %
100 %	5.036 μg	4.905 μg	97.41 %	
150 %	7.554 μg	7.343 μg	97.21 %	

Limit of Detection and Quantification

The limit of detection (LOQ) was found to be 0.45 $\mu\text{g/ml}$ and the limit of quantification was found out to be 1.55 $\mu\text{g/ml}$.

Precision

The intraday and the interday precision studies yielded results with % RSD less than 2 % which indicated excellent precision of the developed method.

Table. 3 Intraday and Interday Precision

Amount $\mu\text{g/ml}$	Intraday Precision % RSD	Interday Precision % RSD
4	0.2075	0.3302
5	0.0968	0.2061
6	0.0797	0.0742

CONCLUSION

A simple, sensitive and reproducible VIS-Spectrophotometric method has been developed for the estimation and quantification of ferulic acid in *Ferula asafoetida* using Folin – Ciocalteu reagent in presence of an alkaline medium. The results obtained from the recovery indicated that there were no interferences from the other constituents. The developed method can be employed for the routine analysis of ferulic acid from the various species of asafoetida. As the Folin – Ciocalteu reagent measures the amount of total phenolic acid it can be used for the standardization of monoherbal preparation containing asafoetida.

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