# Quantification and in vitro release study of trans ferulic acid by UV spectrophotometric method in tablet dosage form.

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**Abstract:** Many potent bioactive compounds serve as antioxidants in the nutraceutical industry and contribute to the prevention of numerous chronic and degenerative diseases. Also, customers have a highly positive outlook on bioactive compounds because of their additional health and wellness benefits. So, demand for such products has increased in the market. Hence, the development of an analytical method for the rapid and inexpensive quantification of ferulic acid in marketed formulations is very important to ensure that consumers obtain ferulic acid, which gives assurance of the purity of the product. So, the UV spectrophotometric method was developed using the wavelength of maximum absorbance for ferulic acid at 320 nm in methanol for the assay study and 310 nm in phosphate buffer with pH 6.8 for the dissolution study. The linearity and range of the proposed method of ferulic acid calibration curve encompass 5–20 µg/ml and 5–25 µg/ml for assay and dissolution studies, respectively. The variability among the repeatability and intermediate precision studies was less than 2% RSD. The ferulic acid recovered was in the range of 99 to 102 percent from the marketed formulation, recommending that the method can be applied to commercial formulations without the interference of the excipients for both studies.

**Conclusion:** The determination of ferulic acid using UV spectroscopy was made easy, accurate, and affordable. The measurement of ferulic acid in commercial formulations and its dissolution study can be done effectively using the aforementioned UV method to ensure the quality of the product and to study the dissolution profile of the ferulic acid in tablet dosage form. The developed UV technique was effectively employed to estimate the ferulic acid content and in vitro release in the marketed formulation**.**

**Keywords:** Ferulic acid, UV-Spectrophotometric method, dissolution study, antioxidant formulation

## 1. Introduction

Research on natural products with therapeutic benefits for consumers has gained prominence in the pharmaceutical, cosmetic, nutraceutical, and food and beverage industries. Many potent bioactive compounds serve as antioxidants in the nutraceutical industry and contribute to the prevention of numerous chronic and degenerative diseases. Also, customers have a highly positive outlook on bioactive compounds because of their additional health and wellness benefits. So, demand for such products has increased in the market. [1]

Ferulic acid (FA) is a phytochemical that is a member of the phenolic group and was first discovered by Austrian scientist Hlasiwetz Barth and chemically named [E]-3-[4-hydroxy-3-methoxy-phenyl] prop-2-enoic acid (Figure No. 1). [2,3] This substance has several biological, pharmacological, and industrial uses, and its presence is linked to anti-inflammatory,[4] antibacterial,[5] anti-allergic,[6] and anti-cancer [7] actions in addition to the prevention of cardiovascular disorders. [8] Additionally, it showed anti-diabetic effects and immunostimulant qualities. [9] It can also reduce nerve cell damage and aid in cell repair. [10] According to reports, ferulic acid is also used to keep green peas colour tone, prevent green tea from turning brown, and stop oxidation from turning bananas black, minimising the microbiological load. [11] Ferulic acid's structure is also similar to that of tyrosine, and it is thought to prevent the production of melanin by inhibiting tyrosine. Human skin can be shielded from UV radiation by ferulic acid (0.5%). Moreover, the ferulic acid ester inhibited the synthesis of melanin, suggesting it might be a good pigmentation inhibitor. [12 -14]

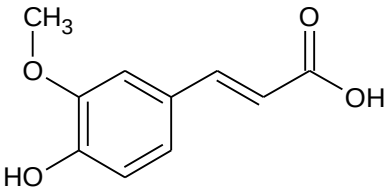


**Figure No. 1: Chemical structure of Ferulic acid**

The trans ferulic acid is preferred by the human body and is not harmful. It has achieved greater attention in countries like the US, Japan, and Korea. Some of the clinical studies have also proven its antihyperlipidemic properties, lowering the risk of cardiovascular disease. [15]

In the coming years, the market for pure ferulic acid is anticipated to grow significantly. Since it can remove free radicals from muscular tissue, ferulic acid supplements are well-liked as sports supplements that can alleviate muscle fatigue. Also, commercially, it is frequently used as a photoprotective agent (sunscreen), a preventative agent against photoaging, and a brightening element in skin care formulations. Hence, its adequate analysis is important to avail its maximum benefit. [16, 17]

Today, various analytical separation and detection techniques have been used for the estimation of ferulic acid. One such is high-performance liquid chromatography (HPLC), along with hesperidin, cinnamic acid, cinnamaldehyde, 6-gingerol, and herbs like asafoetida and ginkgo biloba L-leaves, for ferulic acid quantification. [18-22] Thin-layer chromatography (TLC) along with other bioactive components like gallic acid, caffeic acid, pyrogallolcinnamic acid, etc. [22–24] and capillary tube electrophoresis [25–26] are also reported for ferulic acid estimation. The reported UV spectrophotometric methods are limited to predicting the ferulic acid content only in cereals and herbs. [27–28] Therefore, the development of a new method for the rapid and inexpensive quantification of ferulic acid in marketed formulations is very important to ensure that consumers obtain ferulic acid, which gives assurance of the purity of the product. One of the most widely used methods in pharmaceutical analysis is the UV spectrophotometer. The quantification of the unknown analyte is governed by the Beer-Lambert law in spectrophotometer analysis. Method development is to find out the optimum parameter required for an analytical procedure that will be suitable and sensitive for the analyte quantification. [20]

The objective of this study is to develop an economic and feasible assay and dissolution method to enumerate dissolved ferulic acid in commercially available tablets. Moreover, the quality

control and innovation lab divisions of the nutraceuticals sector might profit from this research study to enhance and regulate the quality of their products.

## 2.Material and method

### 2.1 Materials

Ferulic acid reference standard was procured from Suvidhinath Laboratories Ltd., Vadodara, Gujarat, India. Trans-ferulic acid Antioxidant support 250 mg manufactured by Source Natural was purchased from Amazon India. AR grade Methanol was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. HPLC grade water was procured from Millipore India Ltd, Bangalore, India.

### 2.2 Preparation of standard solution

Correctly weighed 25 mg of ferulic acid reference standard, transferred to a 25-ml volumetric flask, and methanol was added to get 1000 µg/ml. Further, the desired dilutions were made with the selected solvent.

### 2.3 Selection of wavelength

Standard stock solutions of ferulic acid were further pipette separately in a 10 ml volumetric flask with methanol to get different concentration of ferulic acid (5 - 20 µg/ml). Also, standard stock solutions of ferulic acid were further diluted separately with phosphate buffer pH 6.8 to get drug solutions containing 5- 25 µg/ml of ferulic acid concentration. The solutions were scanned in the UV region (200–400 nm), and spectra were recorded for the selection of wavelength.

### 2.4 Analysis of marketed formulation using developed method

Twenty tablets of trans-ferulic acid antioxidant support (250 mg) were accurately weighed, and the average weight was calculated. A quantity equivalent to 0.5 g of ferulic acid from crushed tablets were transferred to a fifty-ml volumetric flask containing 25 ml of methanol. The solution was sonicated, and methanol was added to get a concentration of 10 mg/ml. The resulting sample solution was filtered using Whatman filter paper, and further dilution was performed to get 20 µg/ml of ferulic acid.

### 2.5 Dissolution study

The dissolution tests were carried out in a six-station bath of 900 ml containing phosphate buffer at 6.8 pH. The dissolution apparatus (USP type II) was setup using a paddle with stirring rates of 50 rpm, at 37 °C ± 0.5°C. One tablet of 250 mg ferulic acid was placed in each station, and the temperature was maintained. The amount of withdrawal was 5 ml of dissolution medium at 5, 10, 20, 30, 40, and 45 minute intervals and was replaced with an equal capacity of fresh medium. The collected sample volume was made up to 10 ml with phosphate buffer at 6.8 pH. The solution was filtered through Whatman No. 41 filter paper and analysed spectrophotometrically at 310 nm. A dissolution study was conducted for the commercial formulation of ferulic acid. [29-33]

### 2.6 Drug release kinetics

Drug release data were obtained from the dissolution study was subjected to drug release kinetic study. Data were analyzed using various models like Zero order, First order, Higuchi, Hixon Crowell and KorsemeyerPeppas. After mathematical treatment, model was studied for various

parameters and based on the highest value of regression coefficient, best fit model was chosen for drug release. [34-35]

**2.7 Validation Parameter**: ICH Q2 (R1) guideline was followed to validate the presented method. [36]

**2.7.1 Specificity:**
The presence of ferulic acid unequivocally was demonstrated by performing specificity. A placebo sample of the ferulic acid tablet formulation including excipients such as dibasic calcium phosphate, microcrystalline cellulose, stearic acid, modified cellulose gum, and magnesium stearate was prepared. The assay study and dissolution study was performed by analysing the placebo sample in their specified solvent by the developed method.

**2.7.2 Linearity and range:**
The standard calibration curve was plotted for ferulic acid in the range of 5-20 µg/ml and 5-25 µg/ml in methanol and phosphate buffer (pH 6.8) respectively at their selected wavelength and correlation coefficient was calculated for the described methods. The obtained value of standard deviation of response and mean of slope of the calibration curve was utilized to calculate lowest concentration of detection and lowest concentration of quantification.

**2.7.3 Accuracy:**
Accuracy measures how near the experimental value is to the actual quantity of material in the matrix. So, recovery tests at 50,100,150% level were conducted by measuring the absorbance of spectra at the designated wavelength of the spiked reference drug solution to the pre-analyzed 5µg/ml sample solution of tablet for assay studies.
Ferulic acid reference substance was added to the dissolution vessels in known amounts at the 50%, 100%, and 150% levels along with 250-mg ferulic acid tablet. The dissolution test was performed as described. The resulting solutions were reanalyzed for assay and dissolution studies and % recovery was calculated.

**2.7.4 Precision:**
The level of dispersion is a measure of precision. It is done to prove that an analytical procedure will produce repeatable results at least six times for 15µg/ml of ferulic acid. The intermediate precision of the devised UV technique was established at 20 µg/ml levels of ferulic acid in methanol and % RSD was calculated for assay study.
A dissolution study for six commercial tablets of ferulic acid was performed and statistically RSD of % release of ferulic acid at 45 min was calculated for repeatability study. The intermediate precision was done by finding the RSD (%) of amount of ferulic acid release at 45 minutes for three different days and by three different analysts.

## 3. Results and Discussion

### 3.1 Method development and optimization
Quantitative UV analysis requires the identification of the wavelength of maximal absorption. A solution with an absorbance value less than one is typically regarded adequate for determining the wavelength of maximum absorbance. Taking into account the prerequisites and compatibility, the maximum wavelength for ferulic acid solution (20 µg/ml) was determined

using the full scan mode of a UV-Visible spectrophotometer (Fig. 2). The full scan was analyzed using UV software, and from the spectrum three prominent wavelengths 218 nm, 233 nm and 320 nm were observed. The absorption at 218 nm, 233 nm and 320 nm was found to be linear with respect to concentration. However, 320 nm was adapted as wavelength of measuring in the proposed analytical method to avoid the absorption from excipients and degradation product. Ferulic acid was determined to have wavelengths of maximum absorbance at 320 nm using methanol for assay study as solvent and 310 nm using PBS at pH 6.8 for dissolution study. It is reported that trans ferulic acid undegoes cis isomerization during light exposure, high temperature, high relative humidity (RH> 76%), also, in presence of various formulation excipients. When methanol is used as a solvent, it easily differentiates between the trans and cis isomer of ferulic acid. However, it is reported that hypsochromic shift occur in cis ferulic acid UV spectra compared to trans ferulic acid. (33-34) Solubility of ferulic acid is reported both in methanol and alkaline buffer but for assay studies methanol has to be used as the spectra of ferulic acid is affected by the change in pH. So, methanol was used as a solvent to calculate the purity of ferulic acid in commercial tablet.

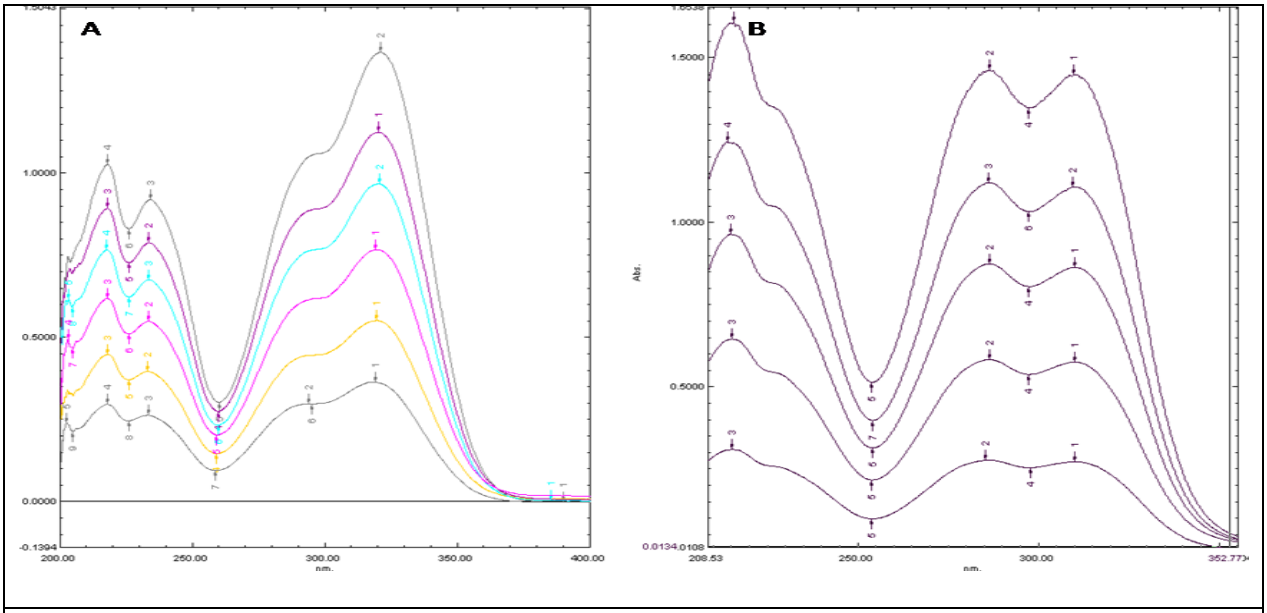


**Figure No 2A Overlain UV spectra of Ferulic acid in methanol (5-20 µg/ml) at detection wavelength 320 nm for assay study.**
**2B Overlain UV spectra of Ferulic acid in phosphate buffer of pH 6.8 (5-25 µg/ml) at detection wavelength 310 nm for dissolution study.**

### 3.2 Preparation of calibration curve

A reliable calibration curve and an equation expressing the link between concentration and response in term of absorbance are required for quantification of unknown samples using a UV-spectrophotometric analysis. Quantitative measurement of Ferulic acid was carried out with the help of a calibration curve which was created utilizing value of absorbance at different concentration. The absorbance of standard solution at 320 nm in methanol and 310 nm in Phosphate buffer at pH 6.8 was measured with a UV spectrophotometer in fixed wavelength mode as shown in Figure 2. Table 1 showed the obtained mean absorbance to plot calibration curve.

**Table 1: Results of calibration curve at detection wavelength 320 nm for assay study and 310 nm for dissolution study.**

| | Linearity data for assay study | | | Linearity data for dissolution study | | |
|---|---|---|---|---|---|---|
| | **Conc. (µg/ml)** | **Absorbance (± SD) *** | **% RSD** | **Conc. (µg/ml)** | **Absorbance (± SD)*** | **% RSD** |
| 1 | 5 | 0.358 ± 0.006 | 1.682 | 5 | 0.276 ± 0.003 | 1.117 |
| 2 | 7.5 | 0.558 ± 0.005 | 1.0001 | 10 | 0.588 ± 0.006 | 1.048 |
| 3 | 10 | 0.770 ± 0.006 | 0.885 | 15 | 0.871 ± 0.007 | 0.852 |
| 4 | 15 | 1.123 ± 0.009 | 0.814 | 20 | 1.138 ± 0.0105 | 0.923 |
| 5 | 20 | 1.516 ± 0.011 | 0.749 | 25 | 1.4642± 0.014 | 0.997 |

*(n= 5), number of determinations, SD (Standard deviation)

### 3.3 Dissolution study of ferulic acid:

The dissolution test for Ferulic acid in commercial tablet formulation has been developed and validated. The 85 % release of ferulic acid was obtained at 35 minutes only as shown in figure no 3.

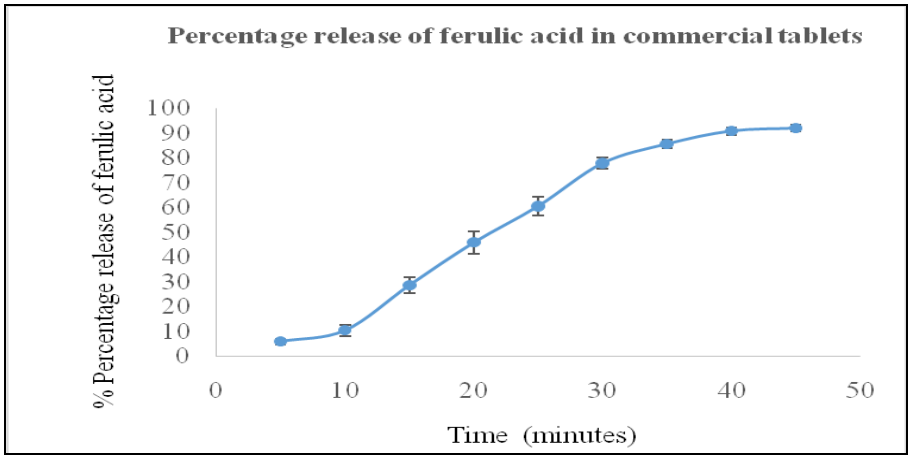


**Figure no 3: In Vitro Drug release of ferulic acid in commercial tablet**

### 3.4 Drug release kinetics:

The following table no. 2 shows the drug release kinetic study. Drug release data was mathematically subjected to various models and analyzed for various parameters. Mathematical analysis indicated that developed formulation follows Higuchi model. The model indicated diffusion-based drug release mechanism from the formulation.

**Table No 2: Kinetic of drug (Ferulic acid) release:**

| Equation parameter | Zero order | First order | Higuchi | Hixson Crowell | Korsmeyer-Peppas |
|---|---|---|---|---|---|
| Slope | 2.437 | 0.029 | 22.42 | 54.50 | 1.529 |
| Intercept | 5.711 | 0.871 | 52.33 | 98.14 | 0.368 |

| | | | | | |
|---|---|---|---|---|---|
| Regression coefficient | 0.957 | 0.833 | 0.968 | 0.960 | 0.961 |

### 3.5 Method Validation

**3.5.1 Specificity:** The specificity was emphasized by the obtained spectrum of assay and dissolution study because there was no interference noted in terms of absorbance at 320 nm and 310 nm respectively.

**3.5.2 Linearity and Range:**
Calibration curve encompassing of 5-20 µg/ml and 5-25 µg/ml in methanol and PBS with pH 6.8 respectively was constructed. Table 1 shows concentration details along with their corresponding mean absorbance values. When the calibration curve values were submitted to least square regression analysis, it produced the following equations: y = 0.1904x + 0.1767 and y = 0.058x - 0.0118 with correlation coefficients of 0.998 and 0.998 for assay and dissolution studies. According to the linearity investigation, the devised UV technique was linear in the pre-defined concentration range of calibration standards.

**3.5.4 Accuracy:**
Accuracy must be ensured over the whole calibration range of the analytical procedure so that findings produced at any point of determination are reliable. Ferulic acid recovery from the preanalyzed formulation was reported to be 99.732 & 100.104 % at 50 %, 100.261 & 99.843 % at 100 % and 101.145 & 99.980 % at 150 % standard addition level of ferulic acid in marketed formulation for both assay and dissolution studies as shown in table no 3.

**Table No 3: Accuracy data of developed UV method for ferulic acid**

| **Recovery Level** | **50%** | **100%** | **150%** |
|---|---|---|---|
| % recovery (mean ± SD)* for assay study | 99.732 ± 1.611 | 100.261 ± 1.510 | 101.145 ± 1.751 |
| % recovery (mean ± SD) * for dissolution study | 100.104 ± 1.409 | 99.843 ± 1.718 | 99.980 ± 1.251 |

(mean ± SD) * (n=3), SD (Standard deviation)

**3.5.5 Precision:**
The relative standard deviation of repeatability and intermediate precision of the devised UV technique of ferulic acid in assay and dissolution study was found to be less than 2 as mentioned in table no 4. Therefore, the described methods will produce repeatable results.

**Table No 4: Precision data of developed UV method for ferulic acid**

| **Parameters** | **Repeatability of measurement (n=6)*** | **Intermediate Precision (n=3)*** | |
|---|---|---|---|
| | | **Different Day** | **Different Analyst** |
| % RSD for Assay study | 0.814 | 0.806 | 0.746 |
| % RSD for dissolution study | 0.852 | 1.001 | 1.003 |

n=number of determinations

**3.5.6 Limit of Quantitation (LOQ) and Limit of Detection (LOD)**

The limit of quantitation (LOQ) is the lowest concentration that can be evaluated with acceptable accuracy and precision. The LOD and LOQ of the suggested UV technique were 0.056 µg/ml and 0.171 µg/ml for assay studies and 0.244 µg/ml and 0.741 µg/ml for dissolution studies. A lower LOQ value showed that the proposed approach would be appropriate for assessing even trace amounts of ferulic acid.

**3.6 Estimation of Ferulic acid in commercial formulation**

The developed UV method was successfully applied for the estimation of 250 mg of ferulic acid content in commercial tablets. The average percent assay of Ferulic acid tablet was found to be 99.153 ± 0.7422 % as given below.

**Table 4: Formulation analysis by developed method**

| **Drugs** | **Amount (mg/Tablet)** | | **% Drug found*** | **% RSD** |
|---|---|---|---|---|
| | **Labeled** | **Found (mean ± SD) *** | | |
| Commercial Tablet of ferulic acid | 250 mg | 247.883 ± 1.855 | 99.153 ± 0.7422 | 0.748 |

(mean ± SD) * (n=6), SD (Standard deviation)

**Conclusion:**

The determination of ferulic acid using UV spectroscopy was made easy, accurate, and affordable. The measurement of ferulic acid in commercial formulations and its dissolution study can be done effectively using the aforementioned method to ensure the quality of product and to study the dissolution profile of the ferulic acid in tablet dosage form. The mentioned developed and validated method can be effectively employed to estimate the ferulic acid content and *in vitro* release in marketed formulation.

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