Quantification and *invitro* 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 nutraceuticals industry and contribute in the prevention of numerous chronic and degenerative diseases**.** Also, customers have a highly positive outlook on bioactive compounds because of its additional health and wellness gains. So their demand of such product is increased in the market. Therefore, the development of a analytical method for the rapid and inexpensive quantification of ferulic acid in marketed formulation is very important to ensure that the consumers obtain the ferulic acid which give assurance of purity of product. So, the UV Spectrophotometric method was developed using the wavelength of maximum absorbance for ferulic acid at 320 nm in methanol for assay study and 310 nm in Phosphate buffer having pH 6.8 for dissolution study. Linearity and range of the proposed method of ferulic acid calibration curve encompassing of 5-20 µg/ml and 5-25 µg/ml for assay and dissolution studies, respectively. Variability among the repetability and intermediate precision study was less than 2 (% RSD). The ferulic acid recovered in ranged of 97 to 102 percent from the marketed formulation, recommending that the method can be applied for commercial formulation without the interference of excipients for both the studies.

Conclusion: The determination of ferulic acid using UV-visible 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-visible method to ensure the quality of product and to study the dissolution profile of the ferulic acid in tablet dosage form. The developed UV-Visible technique was effectively employed to estimate the ferulic acid content and *in vitro* release in marketed formulation.

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

Introduction

Research on natural products with therapeutic benefits to the consumer has gained prominence in pharmaceuticals, cosmetics, nutraceuticals and food and beverage industries [1].

Ferulic acid (FA) (Fig. 1) is a phytochemical that is a member of the phenolic group and was first discovered by Austrian scientist Hlasiwetz Barth and chemically name as ([E]-3-[4-hydroxy-3-methoxy-phenyl] prop-2-enoic acid) (Fig. 1) [2,3]. This substance has several biological, pharmacological, 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 also lessens nerve cell damage and could aid in cell repair[10]. According to reports, ferulic acid keeps green peas' colour tone, prevents green tea from turning brown, and stops oxidation from turning bananas black, minimising 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].

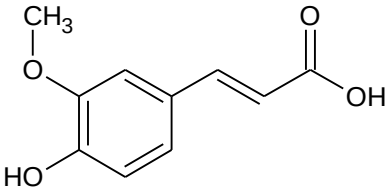

Fig. 1: Chemical structure of Ferulic acid

The trans isoform of ferulic acid is preferred by the human body and is not harmful. It has achieve greater attention in the country like US, Japan and Korea. Some of the clinical studies have also proven its antihyperlipidemia 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 a sports supplement which can alleviate muscle fatigue. Also, commercial it is frequently used as a photo protective 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 technique, such as high-performance liquid chromatography (HPLC) along with hesperidin, cinnamic acid, cinnamaldehyde, 6-gingerol and herbs like asafoetida and ginkgo biloba L-leaves are reported for ferulic acid quantification . [18-22]. Thin-layer chromatography (TLC) along 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 limited to predict 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 formulation is very important to ensure that the consumers obtain the ferulic acid which give assurance of purity of product. One of the most widely used methods in pharmaceutical analysis is UV-visible spectrophotometer. The quantification of the unknown analyte is governed by Beer-Lambert law in spectrophotometric 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 economic and feasible

assay and dissolution method to enumerate dissolved ferulic acid of commercially available tablet. Moreover, the quality control innovation lab divisions of the nutraceuticals sector might profit from this research study to enhance and regulate the quality of their products.

Material and method

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 Amazon. AR grade Methanol was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. HPLC grade water was procured from Millipore India ltd, Bangalore, India.

Method

Preparation of standard solution

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

Selection of wavelength

Standard stock solutions of ferulic acid was further pipette separately in 10 ml volumetric flask with methanol to get 5,7.5,10,12.5,15,17.5,20 µg/ml of ferulic acid. Also, standard stock solutions of ferulic acid was further diluted separately with PBS pH 6.8 to get the drug solutions containing 5,10,15,20,25 µg/ml of ferulic acid. The solutions were scanned in the UV region (200-400 nm) and spectra were recorded.

Preparation of sample solution

Twenty tablets of Trans-ferulic acid antioxidant support 250 mg were accurately weighed and average weight was calculated. Quantity equivalent to 0.5 gm of ferulic acid from crushed tablets was transferred to fifty ml volumetric flask containing 25 ml of methanol. The solution was sonicated and methanol was added to get concentration of 10 mg/ml. The resulting sample solution was filtered using what man filter paper and further dilution was performed to get 20 µg/ml of ferulic acid.

Analysis of marketed formulation using developed method

Market formulation containing ferulic acid was extracted and diluted to get desired concentrations (i.e. 20 µg/ml of ferulic acid) as described earlier in sample preparation. Absorbance was measured and percentage assay was calculated using calibration curve obtained for ferulic acid in methanol

Dissolution study

The dissolution tests were carried out in a six-station bath of 900 ml containing phosphate buffer of 6.8 pH. The dissolution apparatus (USP type II) was setup using a paddle having 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 minutes interval and was replaced with an equal capacity of fresh medium. The collected sample was filtered through Whatman No. 41 filter paper and analyzed spectrophotometrically at 310 nm. Dissolution study was conducted for commercial formulation of ferulic acid. (**29-33)**

Drug release kinetics

Drug release data were obtained from the Dissolution study. Obtained data were subjected to drug release kinetic study. Data were analyzed using various models like Zero order, First order, Higuchi, Hixon Crowell and Korsemeyer Peppas. After mathematical treatment, model was studied for various parameters and based on the highest value of Regression coefficient, best fit model was chosen. **(34-35)**

Validation Parameter: ICH Q2 (R1) guideline was followed to validated presented method **36**.

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 phophate, microcrystalline, cellulose, steraic acid, silica, modified cellulose gum, and magnesium stearate was prepared. The assay study and dissolution study was performed by analysis the placebo sample in their specified solvent by the developed method.

Linearity and range:

The standard calibration curve was plotted for ferulic acid in the range 5-20 µg/ml and 5-25 µg/ml in methanol and PBS with pH 6.8 respectively at their selected wavelength and correlation coefficient was calculated for the described method. 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.

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 5 µg/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 each 250-mg ferulic tablet. The dissolution test was performed as described. The resulting solutions were reanalyzed for assay and dissolution studies and % recovery was calculated.

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 intra- and inter-day precision of the devised UV technique was established at 10, 15, and 20 µg/ml levels of ferulic acid in methanol and % RSD was calculated for assay study.

Dissolution study for six commercial tablet 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 minute for three different day and by three different analyst.

Results and Discussion

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 (20µg/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 wavelength 218 nm, 233 nm and 320 nm were observed. The absorption at 218 nm, 233nm 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 underwent cis isomerization during light exposure, high temperature, high relative humidity (RH> 76%), also, in presence of various formulation excipients. Methanol as solvent was used to calculate the purity of ferulic which can be easily differentiated between cis and trans isomers of ferulic acid as hypsochromic shift occur in cis ferulic acid when compared to trans ferulic acid (**33-34).** The 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.

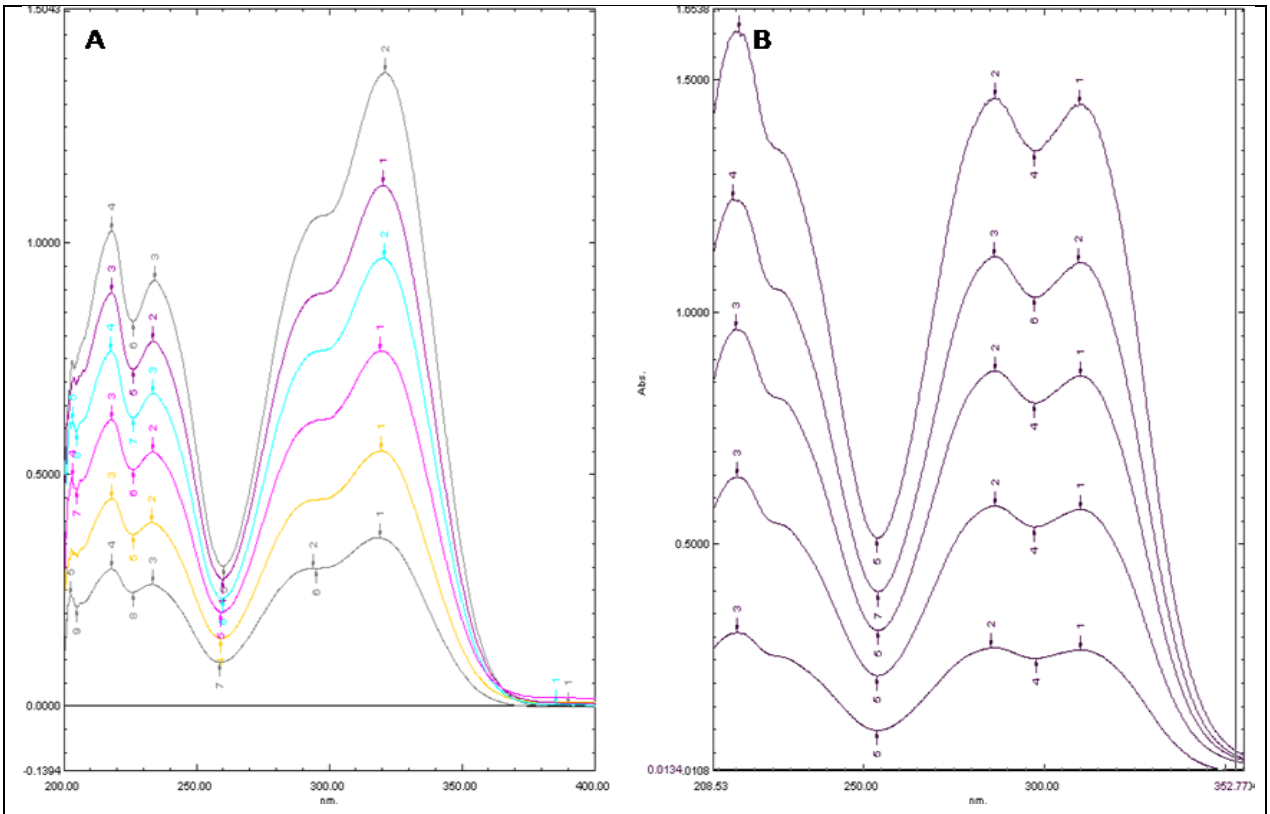

Fig. 2: A Overlain UV spectra of Ferulic acid in methanol (5-20 µg/ml) at detection wavelength 320 nm for assay study.
B Overlain UV spectra of Ferulic acid in phosphate buffer of pH 6.8 (5-25 µg/ml) at detection wavelength 310 nm for dissolution study.

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 Ferulic acid measurement 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-Visible 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 determination, SD (Standard deviation)

Method Validation

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

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 was 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.

Accuracy :

Accuracy measures how near the experimental value is to the actual quantity of measured analyte in the matrix. 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 &100.10% at 50%, 100.26 & 99.84% at 100% and 101.145 & 99.983% at 150% standard addition level of ferulic acid in marketed formulation for both assay and dissolution studies as shown in Table 2.

Table 2: 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 ±1.51	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)

Precision :

The level of dispersion is a measure of 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 3. Therefore, the described methods will produce repeatable results.

Table 3: 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 determination

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

The limit of quantification (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.

Estimation of Ferulic acid

The developed UV method was successfully applied for the estimation of 250 mg of ferulic acid content in commercial tablet. 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)

Dissolution study of ferulic acid:

The dissolution test for Ferulic acid in tablet commercial formulation has been developed and validated. The 85 %release of ferulic acid was obtained at 35 minutes only as shown in figure 3.

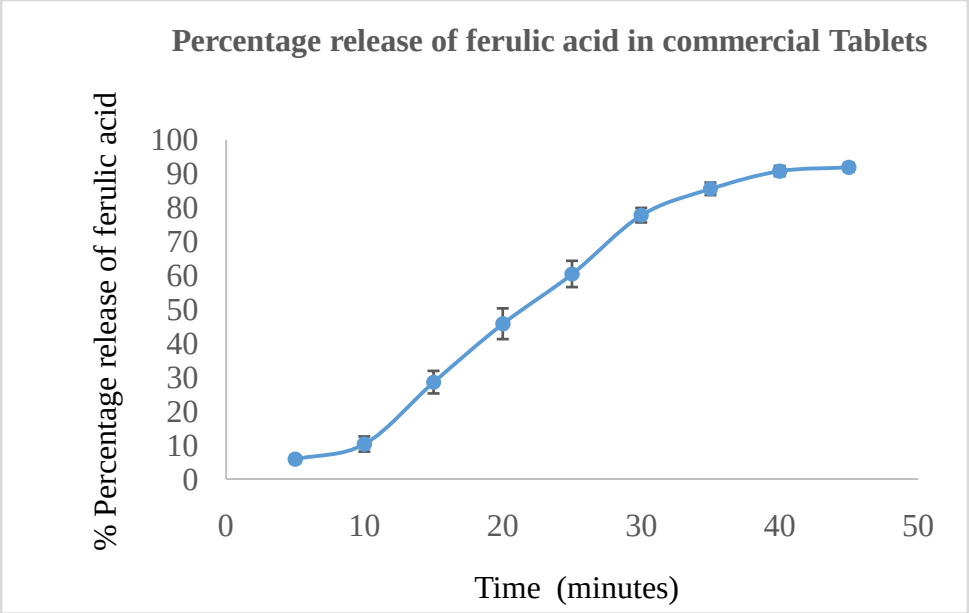

Fig 3: In Vitro Drug release of ferulic acid in commercial Tablet

Following table 5 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 5: Kinetic of drug (Ferulic acid) release:

	Zero order	First order	Higuchi	Hixon crowell	Korsemeyer 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

CONCLUSION: The determination of ferulic acid using UV-Visible 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-Visible method to ensure the quality of product and to study the dissolution profile of the ferulic acid in tablet dosage form. The developed UV-Visible technique can be effectively employed to estimate the ferulic acid content and its *in vitro* release in marketed formulation.

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