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Development of a Ferulic Acid –Enriched Nutraceutical: HPLC Guided Recovery from Crop Waste.

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Abstract

A plentiful agricultural waste product of cereal processing, wheat husk is a key source of phenolic chemicals, particularly ferulic acid, which has strong antioxidant and therapeutic effects. Using a validated HPLC technique, the present goal is to extract and quantitatively assess the ferulic acid in wheat husk. The goal of this research is consistent with current trends in using environmentally friendly and effective analytical methods to transform agricultural waste into high value Nutraceutical components. After alkaline hydrolysis and acidification, ferulic acid was isolated and defatted wheat husk by partitioning with ethyl acetate. The concentrated and filtered extract was screened through chromatography. A high-efficiency RP C18 column (4.6 mm X × 250 mm, 5µmPS) was chosen for the HPLC run and a binary eluent comprising acetonitrile and 0.1% glacial acetic acid was utilized for analyte separation which steadily introduced in column for elution under isocratic conditions. Monitoring of elution was conducted at 320 nm. A sharp and well-resolved ferulic acid peak was observed at a retention time of nearly 6.2 minutes. The validation adhered to ICH Q2 (R1) criteria, and the regression conformity demonstrated across the defined concentration intervals of 50-150µg/mL ($R^2 > 0.99$). Low % RSD demonstrated precision, and percentage recovery varied between 99.5 to 101.1%. In this study, we show how well HPLC can be to analyze ferulic acid from wheat husk, which emphasizes its potential as a functional food ingredient. The technique offers a scalable, reproducible, and environmentally responsible way to valorize agricultural waste into nutraceuticals that improve health.

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Keywords: crop waste, method validation, wheat husk, phenolic compounds, nutraceutical.

1. Introduction

Ferulic acid (FA), a naturally occurring hydroxycinnamic acid, is widely recognized for its multifaceted pharmacological profile, encompassing antioxidant, anti-inflammatory, anti-microbial and anticancer capabilities, making it a valuable compound in Nutraceutical and pharmaceutical applications [1, 2].It is a plant-origin phenolic compound localized in the cell walls of cereals, vegetables, and fruits, with wheat husk being one of the richest and most sustainable sources [3]. In wheat husk, ferulic acid is primarily bound to polysaccharides such as arabinoxylans, limiting its direct bioavailability and requiring effective extraction strategies [4]. Conventional methods of extraction often involve harsh chemicals, extended reaction times, or excessive solvent use, making them inefficient and environmentally burdensome [5]. To overcome these limitations, alkaline hydrolysis followed by organic solvent extraction has been established as a promising method to break the ester bonds and isolate ferulic acid. In Alkaline hydrolysis, ester and ether functionalities undergo cleavage between ferulic acid and arabinoxylan matrix, enhancing its release from the wheat husk structure. The subsequent use of an organic solvent, such as ethyl acetate, efficiently isolates the free ferulic acid for quantification.

This extraction strategy is consistent with the principles of green chemistry, as it reduces hazardous solvent use, promotes the valorization of agricultural waste, and supports environmentally sustainable practices [6]. The structure of ferulic acid ($C_{10}H_{10}O_4$), chemically known as 4-hydroxy-3-methoxycinnamic acid, consists of a substituted aromatic ring with conjugated propionic acid side chain (Figure 1), contributing to its free radical scavenging activity the therapeutic potential.

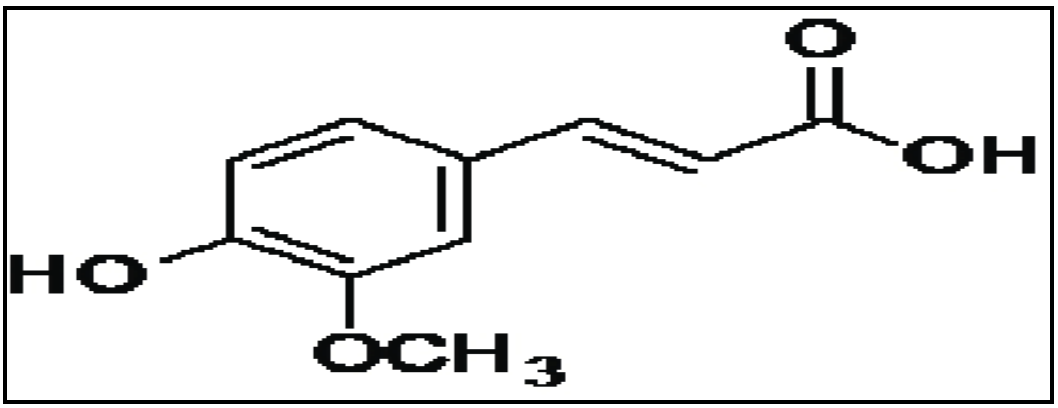

Figure 1: Chemical framework of ferulic acid

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Therefore, the research effort aims to construct and confirm reliability of a green, efficient, and reproducible HPLC method for the quantification of ferulic acid yield from cereal byproducts, ultimately contributing to the sustainable development of nutraceuticals from crop residues.

2. Method

2.1 Materials

Wheat husk was sourced through a local agricultural supplier, cleaned to remove impurities, and stored in a desiccator until use. High Purity Laboratory Chemicals Pvt.Ltd provided a ferulic acid reference standard with a purity of 98%. All of the chemicals and solvents used were of HPLC grade, including Ethyl acetate, glacial acetic acid, acetonitrile and Sodium hydroxide which were purchased from Merk Chemicals. According to GLP, all sample preparations and dilutions were conducted in a typical volumetric flask under regulated laboratory settings.

2.2 Chromatographic Conditions

A high-performance liquid chromatography (HPLC) setup was employed for the chromatographic studies. It was made up of a binary gradient solvent delivery module, an integrated degasser, a manual injection port with a 20µL sample loop, and a UV detector enabled specific monitoring of ferulic acid at its wavelength, ensuring sensitive and accurate quantification. A HiQ Sil C18 HS RP column (250 mm length X 4.6 mm ID, 5 µm particle size) was adopted to attain high-resolution retention profile and clear separation of ferulic acid from complex sample matrices. All analyte solutions underwent filtration using a 0.45µm hydrophilic PTFE film filter before introduction in system to avoid clogging and preserve the integrity of the column's stationary phase. The column temperature was kept at 40°C. To ensure ideal elution and peak shape, the mobile phase was chosen for its volatility and compatibility. It consisted of 0.1% glacial acetic acid in Mili-Q water and HPLC-grade acetonitrile. Employing Lab solutions (Shimadzu), chromatographic elution was performed in gradient mode, delivered at 1.0 mL /min with signal acquisition at 323 nm. This software was responsible for data acquisition, peak integration, and system control, enabling accurate calibration curve production and analytical reproducibility.

Table 1. Programmed Mobile Phase Variation for Enhanced Separation Efficiency

Time (Mins)	**Composition**	
	MP A (%)	**MP B (%)**
0.01	90	10
10.00	90	10
30.00	30	70
35.00	30	70
40.00	90	10
45.00	90	10

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2.3 Preparation of Solution

2.3.1 Composition of Standard Solution:

50 mg of precisely measured ferulic acid was solubilized in 100 mL of methanol (HPLC grade) to create a primary stock solution with a concentration of 500µg/mL. This was done to make the standard solution of ferulic acid. A 2 mL aliquot from this stock was placed in a 10 mL calibrated flask and diluted to volume with methanol to produce working standard solution of 100µg/mL.

2.3.2 Isolation and preparation of Ferulic acid from Wheat husk Matrix.

Accurately weighed 5.0 gm of finely powdered Wheat husk was dispensed in a 250 mL round-bottom vessel. To facilitate the release of bound ferulic acid, 100 mL of 2N sodium hydroxide (NaOH) was added, and the mixture was subjected to alkaline hydrolysis by refluxing at 80°C for 8 hours. After the hydrolysis period, the reaction mixture was brought to ambient temperature under natural cooling conditions, and its pH was carefully adjusted to 2.0 using 6N hydrochloric acid (HCL) to protonate the liberated ferulic acid and its partitioning into the organic solvent. The acidified mixture was filtered through cotton to remove solid residues, and the resulting filtrate was subjected to triple extraction using 100 mL portions of ethyl acetate to ensure complete recovery of ferulic acid. The combined organic layers were pooled and evaporated to dryness under reduced pressure using a rotary evaporator. The remaining residue was redissolved in 10 mL of HPLC-grade Methanol. From this solution, a 2 mL of aliquot was pipetted into a 10 mL standard flask, and the volume was adjusted with methanol to obtain the sample solution. This solution underwent filtration through a 0.45µm porous filter and subsequently loaded into HPLC system for chromatographic analysis.

3. Results

3.1 Method Validation

Ferulic acid that had been taken from wheat husk was successfully quantified using an HPLC technique that was both developed and validated. The approach provided adequate resolution, sensitivity, and reproducibility under ideal chromatographic circumstances. To ensure analytical rigor, the developed method was systematically validated as per regulatory standards. This validated method allows for accurate tracking of the ferulic acid concentration in plant-based matrices and aids in ensuring the quality of phytopharmaceutical preparations [7-9].

3.1.1 Specificity:

To ensure reliable identification of ferulic acid, the specificity showed against potential interferences from wheat husk matrices. As shown in Figure 2, the blank chromatogram exhibited no peaks at the retention time of ferulic acid, indicating no interferences from solvents. Figure 3 presents the graphical image of ferulic acid standard, showing a distinct and well-resolved peak at the expected retention time. Figure 4 illustrates the detector response profile of the wheat husk extract, where the ferulic acid peak appeared at the same retention time as the

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standard, confirming its identity under the optimized chromatographic conditions [10]. No co-eluting or interfering peaks were detected at 323 nm, indicating that the method is capable of selectively quantifying ferulic acid [11]. Furthermore, UV spectral assessment confirmed the purity of the analyte peak, demonstrating the method's ability to distinguish ferulic acid from other phenolic constituents commonly found in cereal matrices [12].

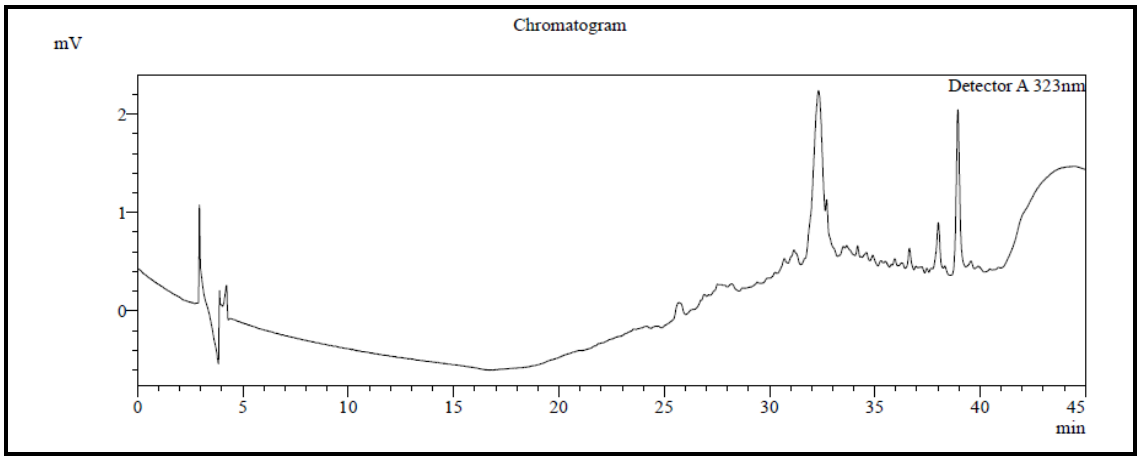

Figure 2. Baseline Response of Blank.

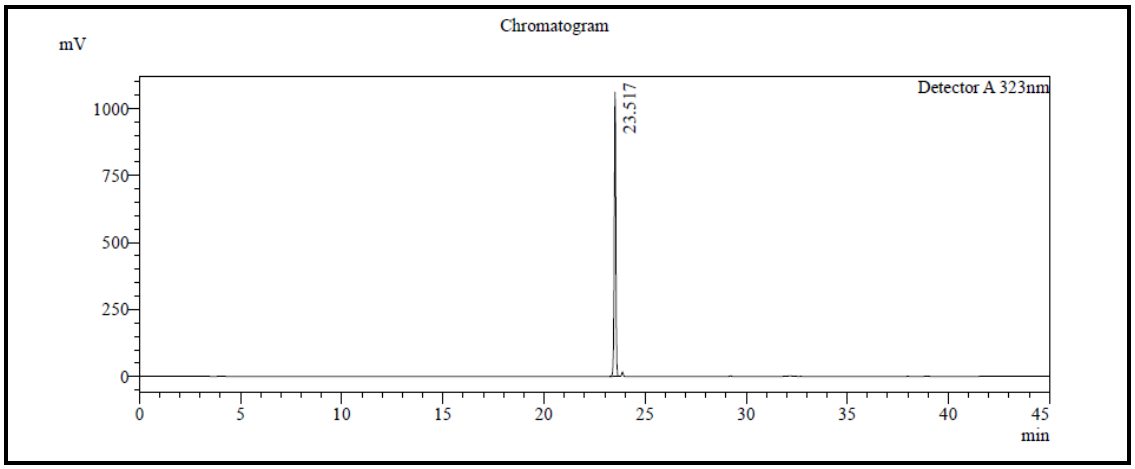

Figure 3. HPLC Elution Profile of Ferulic acid Standard solution

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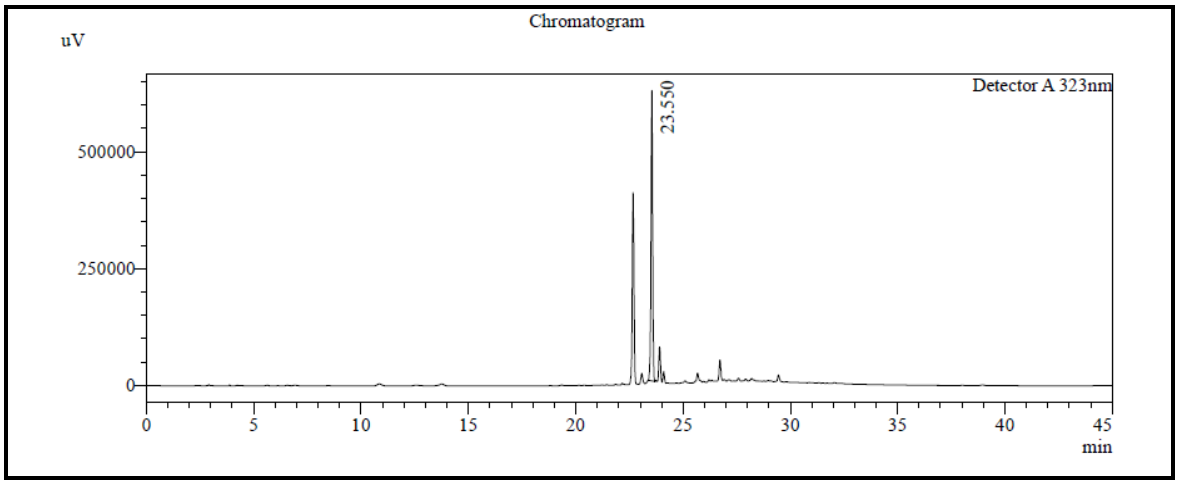

Figure 4. Chromatographic Profile of Ferulic acid from Alkaline Extract of Wheat husk.

3.1.2 Precision

The performance consistency of established HPLC procedure for ferulic acid determination was assessed by evaluating intraday and interday variability. Replicate injections of standard solutions at the same concentrations were analyzed, and the resulting % RSD values remaining below the threshold outlined in ICH recommendations, indicating that the method produces consistent and reproducible results under the tested conditions [13].

Table 2.Precision Evaluation of the developed method for ferulic acid

Parameter	**Sample Solution**	**Ferulic Acid**	
		Content in percentage	**Content in ppm**
Method Precision	Sample Solution-1	0.081	810.81
	Sample Solution-2	0.081	807.41
	Sample Solution-3	0.080	803.44
	Sample Solution-4	0.081	806.83
	Sample Solution-5	0.081	808.23
	Sample Solution-6	0.081	811.95
Intermediate Precision	Sample Solution-1	0.083	832.71
	Sample Solution-2	0.085	848.06
	Sample Solution-3	0.083	825.04
	Sample Solution-4	0.081	813.68
	Sample Solution-5	0.084	843.86
	Sample Solution-6	0.086	857.88
Mean		**0.08**	**816.82**
SD		**0.00**	**14.17**
% RSD		**1.7**	**1.7**

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3.1.3 Linearity

The proportionality between concentration and peak response by preparing ferulic acid standards across a defined concentration range with triplicate assessment conducted for each concentration. The average peak responses were plotted against concentration to construct a standard plot. The method exhibited excellent linearity, with a correlation coefficient (r^2) of 0.9989 across the tested range (10-60µg/mL), confirming a highly proportional response between analyte concentration and detector output [14].

Table 3. Summary of Linearity Data for Analyte

Analyte Concentration	**Average Peak Response**	**SD**	**%RSD**
50	2727723	2003.9	0.07
80	4445407	6393.7	0.14
100	5345577	7456.4	0.14
120	6684230	1193.0	0.02
150	8422300	3970.0	0.05

3.1.4 Accuracy

Overall recovery was determined using pre-extraction spiking approach in which a known quantity of ferulic acid standard was incorporated directly into 5.0 gm of wheat husk before undergoing the full extraction process. The spiked samples at 50%, 100% and 150% levels relative to expected analyte content were allowed to equilibrate, followed by alkaline hydrolysis, liquid-liquid extraction and reconstitution steps identical to those applied to unspiked samples. The detected ferulic acid concentration in the spiked extracts was used to calculate per cent recovery, thereby assessing the combined efficiency of extraction and analysis. This approach provides a robust evaluation of the method's accuracy when applied to complex matrices and aligns with recent HPLC validation practices for phenolic compounds [3].

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Table 4. Accuracy (Recovery) data for Ferulic acid Quantification

Spike Level (%)	Amount Added (in ppm)	Amount Recovered (in ppm)	% Recovery	Mean Recovery (%)	SD	RSD (%)
Acc Level- 50%-1	50.24	50.79	101.10	101.11	0.21	0.21
Acc Level - 50% -2		50.69	100.90			
Acc Level - 50% -3		50.91	101.33			
Acc Level - 100% -1	100.48	101.51	101.03	99.54	1.41	1.41
Acc Level - 100% -2		99.85	99.37			
Acc Level - 100% -3		98.70	98.23			
Acc Level - 150% -1	150.72	151.07	100.23	100.67	0.79	0.79
Acc Level - 150% -2		151.00	100.19			
Acc Level - 150% -3		153.10	101.58			

3.1.5 Robustness

To confirm the method's resilience to slight operational variations, robustness was assessed by applying deliberate minor changes to critical chromatographic parameters, namely, flow rate (±0.1mL/min), monitoring optical band (±2nm) and controlled column temperature (± 2°C). Each modified condition was evaluated using standards and sample solutions. No significant deviation was observed in retention time, peak shape or quantitative results, displaying minimal % RSD and maintaining system suitability criteria. This demonstrates that the method is robust and reliable for ferulic acid analysis under routine conditions [16].

Table 5. Evaluation of Analytical Method Robustness under Slight Experimental Deviations

Parameter Varied	Test Condition	Content (%)	Content (ppm)
Flow Rate	0.9 mL/min	0.082	820.08
	1.1 mL/min	0.083	827.19
Column Oven Temperature	38°C	0.081	812.34
	42°C	0.083	830.81
Detection Wavelength	321 nm	0.082	821.24
	325 nm	0.082	819.34

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3.1.6 Stability Assessment of Solution

Ferulic acid remained stable in liquid phase was evaluated to ensure reliability of the HPLC analysis throughout the experimental period. Standard and sample solutions were formulated in methanol and held at ambient temperature. Aliquots were analyzed at0, 8, 20, 32 and 45 hrs. Using the validated chromatographic method. Stability was determined by monitoring changes in peak area, retention time, and presence of any additional peaks in chromatogram. Solutions were considered stable if the variation in peak area did not exceed ±2% and no degradation peaks were observed. The results indicated that ferulic acid remained stable for at least 45 hrs. These findings are consistent with earlier studies reporting that phenolic acid exhibit good short-term stability in alcoholic solvents, with minimal degradation over 45 hrs under conditions [17].

4. Discussion

With a retention time that matched that of the standard, the established HPLC method produced a well-resolved, sharp, and symmetrical peak for ferulic acid, demonstrating excellent reproducibility and specificity. Additionally, the chromatographic profile of the wheat husk blank extract did not reveal any interfering peaks at the retention time of ferulic acid, supporting peak purity and method selectivity [18].

Calibration data revealed a robust consistent relationship between working levels and peak response over tested range, with a correlation coefficient ($R^2 > 0.99$), demonstrating precise quantification, and the measured ferulic acid content from the wheat husk extract was in close agreement with previously reported values for similar matrices [19]. Recovery studies at multiple spiking levels confirmed within the acceptable range of 98% -102%. Precision studies, including both interday and intraday analyses, yielded %RSD values well below 2%, demonstrating the high repeatability and reproducibility of the method. The approach's sensitivity was demonstrated by the fact that the calculated LOD and LOQ values were low enough to allow for detection and quantification of ferulic acid even in samples with very low concentrations. The method's operational stability and reliability are evidenced by the fact that there was no discernible impact on the retention time, peak area, or resolution during robustness testing under deliberate changes.

In addition to analytical performance, the procedure incorporates environmentally responsible practices by minimizing the use of hazardous solvents, reducing waste production, and optimizing energy usage. Compared with other green extraction approaches, such as microwave-assisted, enzymatic, or pressurized hot water extraction, this method efficiently isolates ferulic acid while valorizing wheat husk, a low-cost agro industrial waste product, into valuable nutraceutical and functional food uses. This agreement with the tenets of green chemistry guarantees that the procedure is not only scientifically sound but also industrially relevant, with the potential for scalability for large-scale production [20].

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5. Conclusion

This work demonstrated that the validated HPLC method is a reliable and effective tool for the determination of ferulic acid content in wheat husk, producing reproducible results that meet the analytical requirements for nutraceutical quality control. The method's precision, linearity, and robustness confirm its suitability for routine use in ensuring consistent bioactive levels in functional food products. Beyond analytical performance, the approach offers notable environmental and economic advantages, including the use of fewer hazardous chemicals, reduced solvent volumes, and compatibility with energy-efficient extraction processes, making it a greener alternative to conventional protocols. These benefits, combined with its straightforward scalability, support its potential adoption in industrial-scale nutraceutical manufacturing. For future application, method optimization could explore integration with advanced green extraction technologies, adaptation to other phenolic-rich agricultural by-products, and establishment of standardized guidelines for its implementation across the nutraceutical sector.

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