

# Method Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Ferulic Acid and Minocycline

King Edward<sup>1\*</sup>, Rajesh Gour<sup>1</sup>, Akhlesh Kumar Singhai<sup>1</sup>

<sup>1</sup>School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh

\*Corresponding author

Date of Submission: 02-05-2026

Date of Acceptance: 11-05-2026

## ABSTRACT

The study was focused on developing and validating a UV spectrophotometric method for the simultaneous estimation of the Ferulic Acid and Minocycline in combined solid dosage forms. Pure drugs were characterized through organoleptic evaluation, solubility testing, and pH determination to ensure their identity and stability. The maximum absorbance wavelengths ( $\lambda_{max}$ ) of each drug were identified, and standard calibration curves were constructed for method development. The analytical procedure was validated in accordance with ICH Q2(R1) guidelines, assessing parameters such as linearity, accuracy, precision, LOD, LOQ, robustness, and reproducibility. In addition, in vitro dissolution studies were carried out to evaluate the release behaviour of both drugs under simulated conditions. The developed method was found to provide a simple, precise, accurate, and reproducible analytical approach suitable for routine quality control and simultaneous estimation of these active pharmaceutical substances.

**Keywords:** Ferulic Acid, Minocycline, UV Spectrophotometry, Dissolution Study, Method Validation, ICH Guidelines.

## I. INTRODUCTION

Analytical chemistry plays a vital role in pharmaceutical research and quality control by providing reliable methods for the identification, characterization, and quantitative estimation of active pharmaceutical ingredients (APIs)(Akash *et al.*, 2025). Among the various analytical techniques available, UV-visible spectrophotometry remains one of the most widely employed methods because of its simplicity, sensitivity, rapidity, accuracy, and cost-effectiveness. Spectrophotometric methods are extensively utilized for routine analysis of pharmaceutical formulations, either individually or in combination dosage forms. The simultaneous estimation of two drugs using spectrophotometry has gained considerable importance in

pharmaceutical industries due to the increasing development of combination therapies and multifunctional formulations(Gupta *et al.*, 2022).

UV-visible spectrophotometry is an analytical technique based on the absorption of ultraviolet or visible radiation by molecules containing chromophoric groups. When electromagnetic radiation passes through a solution containing absorbing species, a portion of the light is absorbed, and the absorbance is directly proportional to the concentration of the analyte according to Beer-Lambert's law(Mandruet *al.*, 2023). The importance of spectrophotometric methods lies in their widespread applicability in the pharmaceutical industry for quality control, drug development, and regulatory compliance. The ability to utilize different reagents to target specific functional groups or chemical structures of drug molecules enhances the versatility of this technique, making it suitable for a broad range of applications, from drug assay to impurity profiling(Verma *et al.*, 2025). Moreover, spectrophotometry offers a non-destructive means of analysis, preserving samples while providing valuable data on concentration, purity, and degradation, thus making it indispensable for routine pharmaceutical analysis. Its significance extends to ensuring drug safety and efficacy, as regulatory agencies require precise analytical methods for drug approval and monitoring throughout the product's lifecycle (Joshi, 2025).

Ferulic Acid is a naturally occurring phenolic phytoconstituent belonging to the hydroxycinnamic acid group. Chemically, it is known as 4-hydroxy-3-methoxycinnamic acid(Singh Tuli *et al.*, 2022). It is widely distributed in plant cell walls and occurs naturally in cereals, rice bran, wheat, oats, fruits, vegetables, and medicinal plants. Ferulic Acid possesses remarkable pharmacological and biological activities due to its strong antioxidant potential. It acts as an effective free radical scavenger and protects biological

systems against oxidative stress-induced damage. The presence of phenolic hydroxyl groups in its structure contributes significantly to its antioxidant activity(Dędek *et al.*, 2019).

Ferulic Acid also improves collagen synthesis and inhibits lipid peroxidation, making it useful in wound healing and anti-aging formulations. The increasing utilization of Ferulic Acid in pharmaceutical preparations necessitates the development of reliable analytical methods for its quantitative estimation(Neopaneet *et al.*, 2023).

Minocycline is a semisynthetic derivative of tetracycline antibiotics. Chemically, it is known as 7-dimethylamino-6-demethyl-6-deoxytetracycline. It possesses broad-spectrum antibacterial activity against both Gram-positive and Gram-negative microorganisms(Fernandes, 2022).Minocycline exerts its antibacterial action by inhibiting bacterial protein synthesis through reversible binding to the 30S ribosomal subunit. This prevents aminoacyl transfer RNA attachment and inhibits peptide chain elongation, thereby suppressing bacterial growth(Anandabaskar, 2021).

Minocycline is particularly effective in acne management because it suppresses bacterial proliferation and reduces inflammatory responses associated with skin lesions. However, the drug is susceptible to degradation by environmental factors such as light, temperature, moisture, and pH changes. Therefore, accurate and validated analytical methods are essential for its quality assessment and stability evaluation(Martins *et al.*, 2021).

The present study is concerned with the development and validation of a UV spectrophotometric method for the simultaneous estimation of Ferulic Acid and Minocycline. Both compounds possess significant pharmacological importance and have been extensively investigated for their therapeutic applications in antimicrobial, antioxidant, anti-inflammatory, dermatological, and wound healing preparations.

## II. MATERIAL AND METHOD

### 2.1 Chemicals

Distilled water was procured from Purensa. DMSO and Methanol was received from Rankem. Ethanol was acquired from Bio LiqaPvt. Ltd. Dichloromethane was procured from Finar.

### 2.2 Preliminary studies

#### 2.2.1 Organoleptic properties

Organoleptic characteristics refer to the physical attributes of substances, including color, odor, and texture(Prasannakumardesuet *et al.*,2015).

#### 2.2.2 pH determination

pH determination is a crucial step in drug analysis and affects solubility and stability. A pH meter was used in the electrometric approach to assess a solution's acidity or alkalinity. [51]

#### 2.2.3 Melting point determination

In the pharmaceutical sector, determining the melting point is essential to maintaining the stability, identity, and purity of Active Pharmaceutical Ingredients (APIs)(John, 2013).

#### 2.3 Solubility Study

Solubility is a fundamental property in analytical chemistry that determines how a compound behaves in different solvents. This approach involved filling a clean, stoppered flask with a fixed volume of solvent and adding a known excess of the drug sample. In order to guarantee adequate mixing and enable the drug to achieve equilibrium with the solvent, the flask was then continually shaken using a mechanical shaker at a regulated temperature. Depending on the type of drug and solvent system, the shaking was carried out for a sufficient period of time, usually 24 to 48 hours. A mixture was allowed to settle after equilibration, and the solution was examined visually (Veselić *et al.*, 2019).

#### 2.4 Identification of pure compound via FTIR spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy is a widely used analytical technique for the identification of organic and inorganic compounds by measuring their absorption of infrared radiation. It helps in detecting functional groups based on characteristic absorption peaks in the infrared spectrum. In this study, FTIR was employed to identify compounds and confirm their molecular structure by observing key functional groups such as –OH, –NH<sub>2</sub>, C=O, and C–H. The spectrum of the test sample was compared with that of a reference standard to ensure purity and to detect any possible impurities(Nehal Upadhyay *et al.*, 2024).

A systematic procedure was followed for FTIR analysis using a Perkin Elmer FTIR spectrometer. The instrument was switched on and allowed to stabilize for 15–30 minutes, followed by a background (air) scan using Spectrum software for calibration. The sample was prepared using the potassium bromide (KBr) pellet method by finely grinding 1–2 mg of the drug with about 100 mg of dry KBr to obtain a uniform mixture. This mixture

was compressed under high pressure using a hydraulic press to form a transparent pellet. The pellet was then placed in the sample holder, and the spectrum was recorded over the range of 4000–400  $\text{cm}^{-1}$ . The obtained IR spectrum was saved for further interpretation and analysis (Devi Datt Joshi, 2012).

## 2.5 Method Development by UV spectroscopy

The principle of UV–visible spectroscopy is based on the absorption of ultraviolet or visible light by chemical molecules, resulting in characteristic spectra. The relationship between absorbance, concentration, and path length follows Beer–Lambert’s law, as shown below:

$$A = a \cdot b \cdot c$$

This study involves key steps in method development, including selection of an appropriate solvent, determination of the maximum wavelength ( $\lambda_{\text{max}}$ ), optimization of instrumental parameters, simultaneous estimation of two drugs, and validation of the developed method in accordance with regulatory guidelines (Mandruet *al.*, 2023).

### 2.5.1 Determination of wavelength of maximum absorbance ( $\lambda_{\text{max}}$ )

A standard solution of Ferulic acid and Minocycline was examined in the ultraviolet range of 200–400nm using a UV–Visible spectrophotometer, with the solvent (methanol) serving as the blank baseline for correction (PrernaWankhede, 2026).

### 2.5.2 Preparation of standard stock solution

A stock solution was prepared by dissolving 10mg of the Ferulic acid and Minocycline separately in a 10ml volumetric flask, followed by dilution to volume with methanol and sonicated.

### 2.5.3 Simultaneous equation method

If a sample contained two absorbing substances, Ferulic acid and Minocycline, each of which absorbed at the maximum absorption wavelength ( $\lambda_{\text{max}}$ ) of the other, it was feasible to ascertain the concentrations of both substances under specific conditions. The absorptivities (molar absorptivities or extinction coefficients) of Ferulic acid at  $\lambda_1$  and  $\lambda_2$ , denoted as  $a_{x1}$  and  $a_{x2}$ , respectively, the absorptivities of substance Minocycline at wavelengths  $\lambda_1$  and  $\lambda_2$ , represented as  $a_{y1}$  and  $a_{y2}$ , respectively, the absorbance of the diluted sample at wavelengths  $\lambda_1$  and  $\lambda_2$ , denoted as  $A_1$  and  $A_2$ , respectively (Lotfyet *al.*, 2020).

Two equations were constructed based on the fact that at  $\lambda_1$ , the absorbance of the mixture is the sum of the individual absorbances of Ferulic acid and Minocycline:

$$C_x = \frac{[(A_2 \cdot a_{y1}) - (A_1 \cdot a_{y2})]}{[(a_{x2} \cdot a_{y1}) - (a_{x1} \cdot a_{y2})]}, \text{ and } C_y = \frac{[(A_1 \cdot a_{x2}) - (A_2 \cdot a_{x1})]}{[(a_{x2} \cdot a_{y1}) - (a_{x1} \cdot a_{y2})]}$$

## 2.6 Method Validation

The process of performing laboratory testing to confirm or ascertain that a system, assay, or procedure produces precise and consistent results within the designated range for the intended analytical application is known as validation (Peris-Vicente *et al.*, 2015).

### 2.6.1 Linearity and range

Linearity of Ferulic acid (2–10  $\mu\text{g/mL}$ ) and Minocycline (4–12  $\mu\text{g/mL}$ ) was evaluated by measuring absorbance at  $\lambda_{\text{max}}$  using methanol as blank. Calibration curves of absorbance versus concentration showed good linearity with a high correlation coefficient ( $R^2$ ), indicating a direct proportional relationship (Moosaviet *al.*, 2018).

### 2.6.2 Precision

Precision refers to the reproducibility of an analytical method and is usually expressed as standard deviation. According to ICH guidelines, it should be evaluated at three levels—repeatability, intermediate precision, and reproducibility (McAlinden *et al.*, 2015).

Repeatability ( $n=6$ ) single, Intraday Precision ( $n=3$ ) for three times in a day, Interday Precision ( $n=3$ ) for three alternative days of selected concentration of Ferulic acid and Minocycline.

### 2.6.3 Robustness

Robustness is the ability of an analytical method to remain consistent under small, deliberate variations in conditions. As per ICH guidelines, it is evaluated by assessing the effect of factors like temperature and other parameters. In this study, robustness was tested by measuring the absorbance of drug solutions at two different temperatures (Ferreira *et al.*, 2017).

### 2.6.4 Ruggedness

Ruggedness indicates the reliability of a method under different conditions such as different analysts. It was evaluated by measuring the absorbance of prepared drug solutions by two different analysts, showing consistent and reproducible results.

### 2.6.5 Detection Limit

The lowest detectable concentration of a drug is called the detection limit. As in concentration range (LOD), an analyte that is detectable but not quantifiable in a sample. According to the formula,

$$\text{LOD} = 3.3 \sigma / S$$

Where,  $\sigma$  = Relative standard deviation of the response, and  $S$  = the slope of the calibration curve (Taleuzzaman, 2018).

### 2.6.6 Quantization limit

The limit of measurement refers to the lowest concentration of the analytical method that can be quantified with sufficient sensitivity and efficiency under laboratory-confirmed conditions of the product. According to the formula,

$$LOQ = 10\sigma / S$$

Where,  $\sigma$  = Relative standard deviation of the response, and S = the slope of the calibration curve (Antonucci *et al.*, 2026).

## III. RESULTS AND DISCUSSION

### 3.1 Preliminary studies of standard drug

#### 3.1.1 Organoleptic properties of Ferulic acid and Minocycline

Table 1: Organoleptic properties of Ferulic acid and Minocycline

Organoleptic Properties	Ferulic acid	Minocycline
Physical appearance	Crystalline powder	Crystalline powder
State	Solid	Solid
Color	White to off- white	Yellow
Odor	Odorless	Odorless

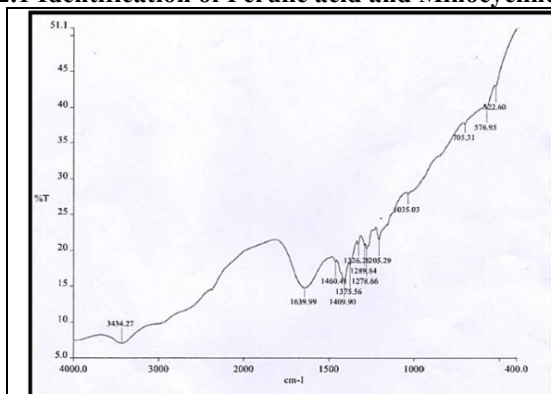
#### 3.1.2 pH and Melting point determination

Table 2: pH and Melting point of Ferulic acid and Minocycline

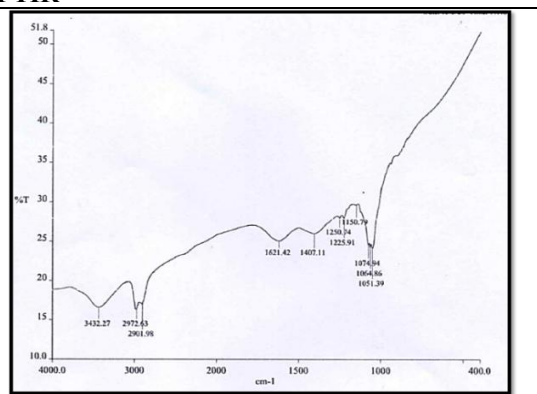
Drug	Reference range (pH)	Observation (pH)	Reference (Melting point)	Observed (Melting point)
Ferulic acid	3-5	5.3	172°C- 174°C	174°C
Minocycline	4.5-5.0	4.8	187°C -193°C	189°C

### 3.2 FTIR

#### 3.2.1 Identification of Ferulic acid and Minocycline by FTIR



Graph 1: FTIR of pure Ferulic acid



Graph 2: FTIR of pure Minocycline

Table 3: FTIR Interpretation of Ferulic acid

Range	Absorbance	Appearance	Bond	Group
3200-3450	3434	Broad	O-H stretching	Alcohol
1650-1750	1639	Broad, strong	C=O stretching	Carboxylic acid
1450-1375	1460	Weak	C-H bending	Methyl group
1400-1350	1409	Sharp, strong	O-H bending	Alcohol
1000-1300	1278	Medium	C-O stretching	Ether
1000-1300	1205	Sharp	C-O stretching	Alcohol
600-1500	1035	Weak	C-C stretching	Alkane

Table 4: FTIR Interpretation of Minocycline

Range	Absorbance	Appearance	Bond	Group
3200-3450	3432	Broad	O-H stretching	Alcohol

2850-2960	2972	Sharp, strong	C-H stretching	Alkane
1650-1750	1621	Sharp, strong	C=O stretching	Carboxylic acid
1450-1375	1407	Broad	O-H bending	Alcohol
1150-1350	1225	Weak	C-N stretching	Amines
1000-1300	1074	Strong, sharp	C-O stretching	Alcohol
600-1500	1051	Strong, sharp	C-C stretching	Alkane

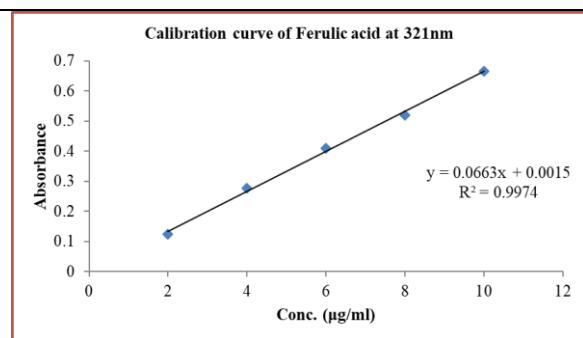
### 3.3 Simultaneous equation method

Table 5: Simultaneous estimation of Ferulic acid and Minocycline

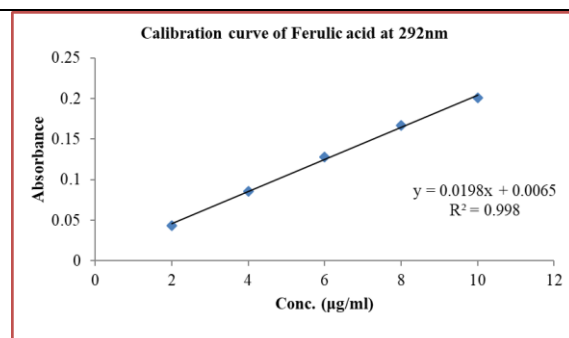
Ferulic acid			Minocycline		
Conc. (µg/ml)	Absorbance		Conc. (µg/ml)	Absorbance	
	321 nm	292nm		321 nm	292nm
2	0.124	0.043	4	0.055	0.097
4	0.277	0.086	6	0.072	0.142
6	0.409	0.127	8	0.090	0.176
8	0.520	0.166	10	0.108	0.220
10	0.665	0.200	12	0.130	0.262

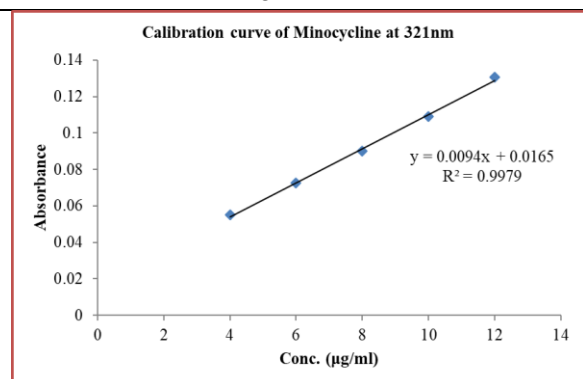
Ferulic acid			Minocycline		
Conc. (µg/ml)	Absorptivity		Conc. (µg/ml)	Absorptivity	
	321 nm	292nm		321 nm	292nm
2	0.062	0.021	4	0.013	0.024
4	0.069	0.021	6	0.012	0.023
6	0.068	0.021	8	0.011	0.022
8	0.065	0.020	10	0.010	0.022
10	0.066	0.020	12	0.010	0.023



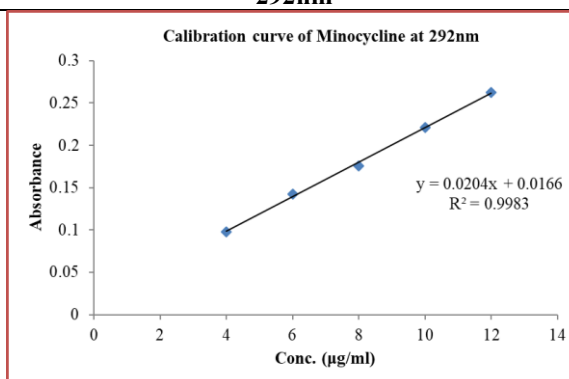
Graph 3: Calibration curve of Ferulic acid at 321nm



Graph 4: Calibration curve of Ferulic acid at 292nm



Graph 5: Calibration curve of Minocycline at 321nm



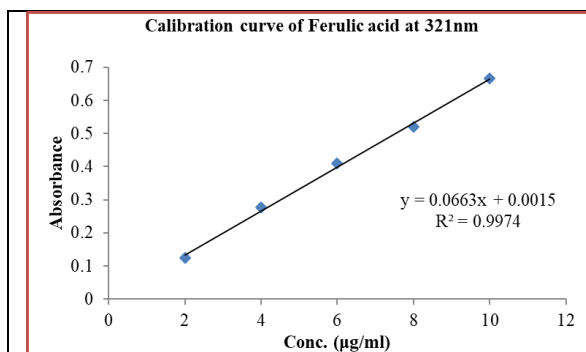
Graph 6: Calibration curve of Minocycline at 292nm

### 3.4 Method validation

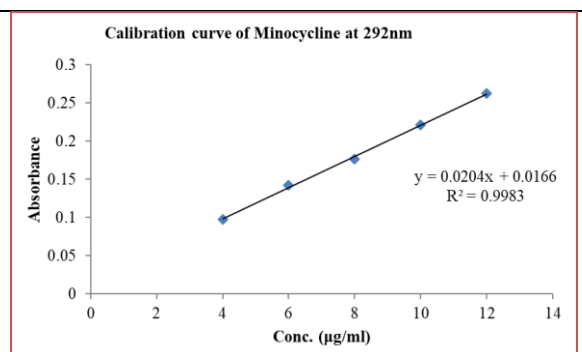
#### 3.4.1 Linearity and range

**Table 6: Calibration data of Ferulic acid at 321nm and Minocycline at 292nm**

Conc. (µg/ml)	Mean Abs	Abs1	Abs2	Abs3	Mean Abs	Abs1	Abs2	Abs3
	Ferulic acid at 321nm				Minocycline at 292nm			
2	0.124	0.123	0.125	0.126	0.097	0.105	0.095	0.091
4	0.277	0.275	0.279	0.277	0.142	0.144	0.146	0.138
6	0.409	0.407	0.408	0.412	0.176	0.179	0.174	0.175
8	0.52	0.521	0.523	0.518	0.220	0.223	0.222	0.216
10	0.665	0.663	0.667	0.666	0.262	0.264	0.263	0.259
<b>Mean</b>	0.399	0.4002	0.3996	0.3998	0.179	0.183	0.18	0.1758
<b>SD</b>	<b>0.0013</b>				<b>0.0036</b>			
<b>%RSD</b>	<b>0.340</b>				<b>2.013</b>			



**Graph 7: Calibration curve of Ferulic acid at 321nm**



**Graph 8: Calibration curve of Minocycline at 292nm**

#### 3.4.2 Precision study

##### 3.4.2.1 Repeatability

**Table 7: Repeatability of Ferulic acid and Minocycline**

Conc. (µg/ml)	Absorbance of Ferulic acid at 321nm	Absorbance of Minocycline at 292nm
4	0.277	0.174
4	0.276	0.176
4	0.279	0.176
4	0.278	0.178
4	0.277	0.179
4	0.279	0.177
<b>Mean</b>	0.277	0.176
<b>SD</b>	0.001	0.001
<b>% RSD</b>	0.436	0.991

##### 3.4.2.2 Intraday Precision

**Table 8: Result of Intraday precision of Ferulic acid and Minocycline**

Concentration (µg/ml)	Absorbance (Morning)	Absorbance (Afternoon)	Absorbance (Evening)	Absorbance (Morning)	Absorbance (Afternoon)	Absorbance (Evening)
	Ferulic acid			Minocycline		
4	0.279	0.276	0.278	0.177	0.176	0.179
4	0.278	0.277	0.277	0.178	0.175	0.178
4	0.278	0.278	0.277	0.178	0.175	0.179
<b>Mean</b>	0.278	0.277	0.277	0.177	0.175	0.178
<b>SD</b>	0.0005	0.001	0.0005	0.0005	0.0005	0.0005

%RSD	0.207	0.361	0.208	0.324	0.329	0.323
AVG % R.S.D	0.258			0.325		

### 3.4.2.3 Interday Precision

Table 9: Result of Interday Precision of Ferulic acid and Minocycline

Concentration (µg/ml)	Day 1 Absorbance	Day 2 Absorbance	Day 3 Absorbance	Day 1 Absorbance	Day 2 Absorbance	Day 3 Absorbance
	Ferulic acid			Minocycline		
4	0.276	0.277	0.280	0.175	0.177	0.179
4	0.276	0.279	0.279	0.176	0.176	0.178
4	0.277	0.278	0.279	0.176	0.177	0.179
Mean	0.276	0.278	0.279	0.175	0.176	0.178
SD	0.0005	0.001	0.0005	0.0005	0.0005	0.0005
%RSD	0.208	0.359	0.206	0.328	0.326	0.323
AVG % R.S.D	0.258			0.326		

### 3.4.3 Ruggedness

Table 10: Result of ruggedness of Ferulic acid and Minocycline

Conc (µg/ml)	Analyst 1 Absorbance	Analyst 2 Absorbance	Analyst-1 Absorbance	Analyst-2 Absorbance
4	0.276	0.280	0.175	0.178
4	0.277	0.281	0.176	0.179
4	0.277	0.280	0.175	0.179
Mean	0.276	0.280	0.175	0.178
SD	0.0005	0.0005	0.0005	0.0005
%RSD	0.208	0.205	0.329	0.323

### 3.4.4 Robustness

Table 11: Results showing robustness of Ferulic acid and Minocycline

Concentration (µg/ml)	Absorbance at 10°C	Absorbance at 35°C	Absorbance at 10°C	Absorbance at 35°C
4	0.274	0.278	0.174	0.179
4	0.273	0.279	0.176	0.179
4	0.273	0.279	0.176	0.178
Mean	0.273	0.278	0.175	0.178
SD	0.0005	0.0005	0.001	0.0005
% RSD	0.211	0.207	0.658	0.323

### 3.4.5 LOD and LOQ of Ferulic acid and Minocycline

Table 12: Results showing LOD and LOQ of Ferulic acid and Minocycline

Drug name	Wavelength	LOQ (µg/ml)	LOD (µg/ml)
Ferulic acid	321nm	1.96	0.64
Minocycline	292nm	1.76	0.582

Table 13: Optical Characteristics and Validation Study of Drugs

Parameters	Ferulic acid	Minocycline
Wavelength λ max nm	321nm	292nm
Beer's law limit µg/ml	2-10	4-12
Correlation coefficient (R <sup>2</sup> )	0.997	0.998
Slope	0.066	0.020
Intercept	0.001	0.016
SD	0.0013	0.0036
% RSD	0.340	2.013

<b>Precision</b>		
<b>Repeatability</b>	0.436	0.991
<b>Intraday (% RSD)</b>	0.258	0.325
<b>Interday (% RSD)</b>	0.258	0.326
<b>Ruggedness</b>		
<b>Analyst 1 (% RSD)</b>	0.208	0.329
<b>Analyst 2 (% RSD)</b>	0.205	0.323
<b>Robustness</b>		
<b>Temp. 10°C (% RSD)</b>	0.211	0.658
<b>Temp. 35°C (% RSD)</b>	0.207	0.323
<b>LOQ (µg/ml)</b>	1.96	1.8
<b>LOD (µg/ml)</b>	0.64	0.594

### Discussion

The study successfully confirmed the identity and purity of Ferulic acid and Minocycline through organoleptic evaluation, pH, and melting point analysis, all of which were within standard reference limits. FTIR spectral analysis verified the presence of characteristic functional groups, supporting the structural integrity of both compounds. UV-visible spectroscopy revealed distinct  $\lambda_{max}$  values with excellent linearity ( $R^2 \approx 0.997-0.998$ ), confirming adherence to Beer-Lambert's law and suitability for quantitative analysis. The developed method demonstrated high sensitivity, with low LOD and LOQ values, particularly for Minocycline, indicating reliable detection at low concentrations. Precision studies showed excellent repeatability, intraday, and interday consistency (%RSD < 2%), confirming the reliability of the method. Ruggedness evaluation across different analysts indicated reproducibility, while robustness studies under varying temperature conditions (10°C and 35°C) confirmed method stability. Overall, the analytical method proved to be accurate, precise, sensitive, robust, and suitable for simultaneous estimation of both drugs in pharmaceutical analysis.

### IV. CONCLUSION

The study was concluded by establishing that the developed UV-spectrophotometric method was simple, accurate, precise, and reliable for the simultaneous estimation of ferulic acid and minocycline in pharmaceutical formulations. The method was validated according to ICH guidelines, ensuring that parameters such as accuracy, precision, linearity, LOD, LOQ, ruggedness, and robustness were within acceptable limits. It was demonstrated that the method was applicable for routine quality control analysis of combined drug dosage forms. Overall, the results confirmed that this method was cost-effective, reproducible, and

suitable for practical analytical and formulation studies in the pharmaceutical field.

### REFERENCES

- [1]. Prasannakumardesu, G Vaishnavi, U Lakshmi, K Divya. An Overview on Preformulation Studies. IAJPS. 2015, 2 (10), 1399-1407
- [2]. John C. O'C. Young. True Melting Point Determination. Chem. Educator 2013, 18, 203-208.
- [3]. Veseli A, Žakelj S, Kristl A. A review of methods for solubility determination in biopharmaceutical drug characterization. Drug development and industrial pharmacy. 2019 Nov 2;45(11):1717-24.
- [4]. Nehal Upadhyay, Prof. Mitali Dalwadi, Dr. Umesh Upadhyay. UV Visible Spectroscopy Method Development and Validation. IJARESM. June-2024, Volume 12, Issue 6, 2455-6211.
- [5]. Devi Datt Joshi. FTIR Spectroscopy: Herbal Drugs and Fingerprints. Springer, India. January 2012.
- [6]. Mandru A, Mane J, Mandapati R. A review on UV-visible spectroscopy. Journal of Pharma Insights and Research. 2023 Dec 1;1(2):091-6.
- [7]. Purna Wankhede. Development And Validation of A UV Spectrophotometric Method for the Assay of Buspirone HCL Tablets. Int. J. of Pharm. Sci., 2026, Vol 4, Issue 2, 3666-3673.
- [8]. Lotfy HM, Fayez YM, Michael AM, Monir HH, Nessim CK. Paired wavelength relevance as spectrophotometric strategy for evaluation the potency of medicine affecting human health. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020 Oct 5;239:118461.

- [9]. Peris-Vicente J, Esteve-Romero J, Carda-Broch S. Validation of analytical methods based on chromatographic techniques: An overview. *Analytical separation science*. 2015 Dec 7:1757-808.
- [10]. Moosavi SM, Ghassabian S. Linearity of calibration curves for analytical methods: a review of criteria for assessment of method. *Calibration and validation of analytical methods: a sampling of current approaches*. 2018 Apr 25;109.
- [11]. McAlinden C, Khadka J, Pesudovs K. Precision (repeatability and reproducibility) studies and sample-size calculation. *Journal of Cataract & Refractive Surgery*. 2015 Dec 1;41(12):2598-604.
- [12]. Ferreira SL, Caires AO, Borges TD, Lima AM, Silva LO, dos Santos WN. Robustness evaluation in analytical methods optimized using experimental designs. *Microchemical Journal*. 2017 Mar 1;131:163-9.
- [13]. Taleuzzaman M. Limit of blank (LOB), limit of detection (LOD), and limit of quantification (LOQ). *Org. Med. Chem. Int. J.* 2018;7(5):127-31.
- [14]. Antonucci A, Albano M, Pindinello I, Fabiani L, Cipollone C, Mastrangeli G, Mastrantonio R, Muselli M, Ursini CL, Cavallo D, Petyx M. A new tool to support the protection of workers' health: development and application of an innovative analytical method for biomonitoring occupational exposure to formaldehyde. *Analytical and Bioanalytical Chemistry*. 2026 Mar 31:1-4.
- [15]. Akash MS, Rehman K. Comprehensive insights into pharmaceutical analysis. In *Essentials of Pharmaceutical Analysis 2025* Apr 30 (pp. 1-62). Singapore: Springer Nature Singapore.
- [16]. Gupta D, Bhardwaj S, Sethi S, Pramanik S, Das DK, Kumar R, Singh PP, Vashistha VK. Simultaneous spectrophotometric determination of drug components from their dosage formulations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2022 Apr 5;270:120819.
- [17]. Mandru A, Mane J, Mandapati R. A review on UV-visible spectroscopy. *Journal of Pharma Insights and Research*. 2023 Dec 1;1(2):091-6.
- [18]. Verma A, Tiwari MR. Applications Of Spectroscopy In Pharmaceutical Analysis And Quality Control. *Journal of Pharmaceutical Analysis and Drug Research*. 2025 Oct 13;7(2).
- [19]. Joshi NK. Advanced Spectroscopic Techniques in Pharmaceutical Analysis: A Critical Evaluation. *Journal of Pharmaceutical Analysis and Drug Research*. 2025 Oct 13;7(1).
- [20]. Singh Tuli H, Kumar A, Ramniwas S, Coudhary R, Aggarwal D, Kumar M, Sharma U, ChaturvediParashar N, Haque S, Sak K. Ferulic acid: a natural phenol that inhibits neoplastic events through modulation of oncogenic signaling. *Molecules*. 2022 Nov 7;27(21):7653.
- [21]. Dędek K, Rosicka-Kaczmarek J, Nebesny E, Kowalska G. Characteristics and biological properties of ferulic acid. *Biotechnology and Food Science*. 2019;83(1).
- [22]. Neopane D, Ansari VA, Singh A. Ferulic acid: signaling pathways in aging. *Drug research*. 2023 Jul;73(06):318-24.
- [23]. Fernandes GJ. *The mechanisms underlying minocycline non-antibiotic effects* (Master's thesis, Universidade de Lisboa (Portugal)) 2022.
- [24]. Anandabaskar N. Protein synthesis inhibitors. In *Introduction to basics of pharmacology and toxicology: volume 2: essentials of systemic pharmacology: from principles to practice* 2021 Mar 14 (pp. 835-868). Singapore: Springer Nature Singapore.
- [25]. Martins AM, Marto JM, Johnson JL, Graber EM. A review of systemic minocycline side effects and topical minocycline as a safer alternative for treating acne and rosacea. *Antibiotics*. 2021 Jun 22;10(7):757.