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Preformulation Studies And Simultaneous Estimation Of Fusidic Acid And Diclofenac

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ABSTRACT

Aim: This study aims to perform comprehensive Preformulation studies and develop a validated analytical method for the simultaneous estimation of Fusidic Acid and Diclofenac. These two drugs, widely used in dermatological and anti-inflammatory therapies, require precise formulation strategies to ensure stability, efficacy, and patient safety.

Method: The Preformulation studies included evaluating the physicochemical properties of Fusidic Acid and Diclofenac, such as solubility, pH stability, and thermal stability. Drug-excipient compatibility was assessed using Fourier-transform infrared spectroscopy (FTIR). For simultaneous estimation, a UV-Visible spectrophotometric method and a High-Performance Liquid Chromatography (HPLC) method were developed and validated in accordance with ICH guidelines. Parameters such as linearity, accuracy, precision, and robustness were evaluated.

Results: Fusidic Acid and Diclofenac exhibited distinct solubility profiles, with optimal solubility observed in specific pH environments. Both drugs were found to be stable under various temperature conditions. The FTIR studies confirmed no significant interactions between the drugs and selected excipients, indicating compatibility. The HPLC method developed showed excellent linearity over the concentration range of 5-60 µg/mL for both drugs, with correlation coefficients (R^2) greater than 0.998. The method was precise, with intra- and inter-day variability below 2%.

Conclusion: The Preformulation studies provided critical insights into the physicochemical properties and stability of Fusidic Acid and Diclofenac, facilitating their co-formulation. The validated HPLC method proved to be a reliable tool for the simultaneous estimation of these drugs in combination formulations, supporting further development of combination therapies. These findings pave the way for the formulation of effective and stable pharmaceutical products containing Fusidic Acid and Diclofenac.

Keywords: Fusidic Acid, Diclofenac, Preformulation, HPLC, Simultaneous estimation

1. INTRODUCTION

Preformulation studies are a critical phase in the pharmaceutical development process, serving as the foundational step toward the successful formulation of drug products **(Lau, 2001; Brahmankar and Jaiswal, 2019)**. These studies involve a comprehensive investigation of the physical and chemical properties of drug substances, such as solubility, stability, and compatibility with excipients, which are essential for ensuring the efficacy, safety, and quality of the final pharmaceutical product **(Vilegave et al., 2013; Lachman et al., 1976; Chatwal, 2022).** Fusidic acid (FA), a steroidal chemical bacteriostatic agent, is advised for the treatment of eye infections and both primary and secondary skin illnesses **(Wadhwa et al., 2016).** While many diseases caused by bacteria that exhibit multiple resistances might significantly impair the pharmacological effectiveness of FA, FA is distinguished by its limited spectrum bacteriostatic activity (Gram-positive bacteria). There are several risks and complications connected with each of the dose forms—oral, parenteral, and topical—those are available for it **(Almostafa et al., 2022)**. Fusidic acid, a bacteriostatic agent from *Fusidium coccineum*, is a narrow-spectrum steroid antibiotic primarily active against gram-positive bacteria, including *Staphylococcus aureus, S. epidermis, Clostridium spp.*, and *corynebacterial*. It inhibits protein synthesis in bacteria through four phases: initiation, elongation, translocation, and release. Its four proteins, IF-2, EF-Tu, EF-G, and RRF, have GTPase activity **(Godtfredsen et al., 1962; Biedenbach et al., 2010; Corey, 2009; Lode, 2009; Jyoti et al., 2020)**. Diclofenac diethylamine (DDEA), also known as 2-[2-(2,6-dichloroanilino) phenyl acetic acid or C18H23Cl2N2O2), is a non-steroidal anti-inflammatory medication (NSAID) class of active pharmaceutical ingredient. It is used to treat inflammation brought on by these disorders, low back pain, musculoskeletal issues, strains and sprains, arthritis, contusions (bruises), and post-traumatic pain **(Yuan et al., 2021).** It works by preventing the cyclo-oxygenase (COX) enzyme from producing prostaglandin (PG), a chemical messenger. Less PGs form COX blockage as a result, which aids in lowering pain and inflammation **(Djordjevic et al., 2005).** Fusidic Acid, an antibiotic, and Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), are commonly used in combination therapies, particularly in topical formulations, to treat bacterial infections and

inflammation **(Khichariyaa et al., 2022)**. The preformulation studies in this context are crucial to understand the individual and combined behavior of these APIs, particularly in terms of their solubility, stability, and potential interactions, which directly influence the formulation design and therapeutic efficacy **(Gopinath and Naidu, 2011; Verma and Mishra, 2016; Patel, 2019).** Furthermore, pharmaceutical quantitative method research is critical in monitoring and correctly quantifying pharmaceutical residues in the environment **(Dong et al., 2023).** The simultaneous estimation of these drugs is essential for quality control and ensures accurate dosing in combination products **(Gondalia et al., 2010)**. The analytical methods developed during these studies, often utilizing sophisticated techniques such as high-performance liquid chromatography (HPLC), must be precise, sensitive, and robust to meet the stringent requirements of pharmaceutical analysis **(Shah et al., 2019)**. This research not only aims to optimize the formulation of Fusidic Acid and Diclofenac but also contributes valuable insights into the challenges and considerations involved in the development of multi-drug products, a growing area of interest in pharmaceutical sciences

2. MATERIAL AND METHOD

2.1 Pre-formulation studies of Fusidic acid and Diclofenac

2.1.1 Organoleptic Properties

Organoleptic test or commonly called as sensory test is the way of testing by using the human senses as the main tool for measuring the acceptability of the product consisting of texture, color, shape, aroma, taste of the product. Organoleptic properties were observed by visual observation. The organoleptic studies of Fusidic acid and Diclofenac such as appearance, color, odor, state etc. were observed

2.1.2 Solubility study

A solid, liquid, or gaseous chemical component known as a solute has the ability to dissolve in a solid, liquid, or gaseous solvent to produce a homogenous solution of the solute in the solvent. This feature is known as solubility. Temperature, pressure, and the solvent being employed all have a basic impact on a substance's solubility. The USP NF, 2007 was followed in determining Fusidic acid and Diclofenac qualitative solubility in various solvents. The drug (1 mg) was precisely weighed and put into a 10 ml test tube. It was then dissolved in various combinations of methanol, ethanol, DMSO, water, chloroform, acetone, and n-hexane.

2.1.3 Melting Point

Melting point analysis was done using a digital melting point device in that the thread band was used to secure the capillary to the thermometer. Raising the oil bath's temperature to 110°C will cause urea to melt roughly 20° below its stated melting point of 132°C. The capillary tube and thermometer should be inserted into the oil bath. Keeping an eye on the capillary, heat the bath to roughly 125°C at a rate of 5°C/min. The sample's melting point was determined by noting the temperature at which the sample begins to melt.

2.1.4 PH

Using a digital pH meter, pH was measured (EI). A digital pH meter was used to measure the pH after a medication (Fusidic acid and Diclofenac) of 1-2 mg was dissolved in 10 ml of distilled water. The electrode should be carefully cleaned with deionized water and dried with scientific wipes to prevent dilution of the sample being analyzed. This can be done with the aid of a digital pH meter. The electrode should then be submerged in the solution before being read. pH meters should ideally be stored in an appropriate solution after use.

2.1.5. Partition coefficients

Partition coefficient (oil/ water) is an indicator of drug lipophilicity. The partition coefficient was determined by the shake flask method using two immiscible solvents, the most common hydrophilic solvent was water and octanol as oil phase were taken for the study. A partition coefficient was calculated by the ratio of the concentration of a substance in one medium or phase (C1) to the concentration in a second phase (C2) when the two concentrations are at equilibrium; that is,

Partition coefficient = (C1/C2) equil

2.2 UV estimation For Fusidic acid and Diclofenac

Preparation of standard stock solution:

According to Indian Pharmacopoeia standard stock solution for API were prepared for that 10 mg of Fusidic acid was dissolve in 100 ml of methanol (100 µg/mL). Out of this stock 1-6 ml was pipetted and diluted up to 10 ml by solvent methanol (10-60 µg/mL) and examined between 200-800 nm and 10 mg of Diclofenac was dissolve in 100 ml of methanol (100 µg/mL). Out of this stock 0.5- 3 ml was pipetted and diluted up to 10 ml by methanol (5-30µg/mL) and examined between 200-800 nm. The maximum absorbance was determined using UV-Vis Spectrophotometer (UV- 1700, Shimadzu, Japan) to confirm the λ max of the drugs.

2.2.1 Drug: drug interference study
Standard stock solution (100 µg/ml) of Fusidic acid and Diclofenac were prepared separately in methanol by serial dilution technique. The absorbance values for Fusidic acid and Diclofenac were recorded at 253 nm and 249 nm respectively, using methanol as a blank. Absorptivity values a (1%. 1 cm) were calculated for both wavelengths from absorbance values.

2.2.2 Simultaneous Equation Method
From the standard stock solutions both drug (100 µg/mL), were taken and made it to final concentration of 5-35 µg/ml (Diclofenac) and 10-60 µg/ml (fusidic acid). Absorbance was measured at both the wavelengths by using solvent as blank. The reading was taken in triplicate. Absorbance maxima of both the drugs were recorded at both the wavelengths. The concentration was determined by using simultaneous equation method.

2.2.3 Simultaneous equation method formula
By using the below equations the concentrations in the samples were obtained
CX= A1ay2 – A2ay1 / ax1ay2 – ax2ay1 Eq. 1
CY = A1ax2 – A2ax1 / ay1ax2 – ay2ax1 Eq. 2
where A1 and A2 are absorbances of mixture at 252 nm and 282 nm respectively, ax1 and ax2 are absorptivities of Fusidic acid at λ1 and λ2 respectively, ay1 and ay2 are absorptivities of Diclofenac at λ1 and λ2 respectively, Cx and Cy are concentrations of Fusidic acid and Diclofenac respectively.

2.2.4 Study of Beer's Lambert Law
The solutions having concentrations in range 5-35 µg/ml and 10-60 µg/ml for both Fusidic acid and Diclofenac were prepared in methanol using working standard solution. The absorbances of resulting solutions were measured at 252 nm and 282 nm. Calibration curves were plotted at these wavelengths. Both the drugs obeyed linearity individually and combination within the concentration range of 5-35 µg/ml and 10-60 µg/ml for both drug.

2.2.5 Precision Study (validation for method during analysis)
Both Inter- day precision and Intra-day precision were carried out as per the statistical requirement to support reproducibility of the method.

2.2.5.1 Intra Day Assay (validation for method during analysis)
The assay procedure was carried out in the same day in the duration of 2 hours to 3 hours at fixed concentration and the results were compared.

2.2.5.2 Inter Day Assay (validation for method during analysis)
The assay procedure was carried out in the after day in the duration of 24 hours at fixed concentration and the results were compared.

2.2.6 Ruggedness study (validation for method during analysis)
The ruggedness of the method was determined by carrying out the analysis using two different analysts and the respective absorbance was noted. Ruggedness of the methods was assessed by carrying out assay 6 reading with different analyst by using same equipment.

2.2.7 Robustness study (validation for method during analysis)
To determine the robustness, the same procedure was carried out by changing the temperature and the result is compared with the same previous procedure.

2.3 Fourier transmission Infra-Red Spectroscopy
FT-IR spectrum of Drug and excipient combination was recorded over the range of 4000 to 400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of each drug and drug + polymers in 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 region.

2.4 HPLC GRAPH
2.4.1 Instrumentation
The HPLC system (Waters, Milford, MA, USA) was consisted of a 600 controller pump, a multiple- wavelength PDA detector, an in-line AF 2489 series degasser, a rheodyne 7725i injector with a 20 ml loop with integrated Empower integration software. The separation was performed using C18 100 Å, 150× 3.9mm filled with 5 mm particles (Phenomenex, Torrance, CA, USA) column.

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2.4.2 Chromatographic conditions

The assay of Fusidic acid and diclofenac was performed using externally standardized isocratic conditions. The separation was carried out using the mobile phase consisted acetonitrile and water (80:20, v/v) which was degassed and filtered before run the column. The column temperature was maintained at 25°C and each injection volume was 20 □l. The wavelength was set at 252 nm for Fusidic acid and282nm for diclofenac with a flow rate of 1ml/min and the run time was set at 15 min. The peak identification and retention time (RT) of the sample.

2.4.3 Sample Preparation

Both the drug weighed 1 mg and was dissolved in mobile phase solution to prepare 1 mg/ ml solutions. The standard solution was subsequently diluted to prepared different concentrations 5-30 □g /ml. Mobile phase and dilution primarily filtered with the help of micron filter (0.42 micron) and syringe filter(0.22 micron).After preparation of sample degassed with the help of sonicater and this was performed before analysis.

3. RESULTS AND DISCUSSION

3.1 Preformulation studies of Fusidic acid and Diclofenac

3.1.1 Organoleptic properties

Table 1: Organoleptic properties of Fusidic acid

Drug	Organoleptic properties	Observation
Fusidic acid	Color	White
	Odor	Odourless
	Appearance	Powder
	State	Solid powder
Diclofenac	Color	White
	Odor	Odourless
	Appearance	Powder
	State	Fine powder

An evaluation of the API's organoleptic qualities, including Appearance, color, odor, and state, was conducted. Fusidic acid and Diclofenac were discovered to have a white color to it when tested. Fusidic acid was odourless and has a solid state powder form and Diclofenac was odourless and has a solid state fine powder form, according to research conducted on it. Fusidic acid and Diclofenac exhibited the same appearance, color, odor and state as the I.P. requirements for these characteristics. Result show in Table1.

3.1.2 Solubility study

Table 2: Solubility study of Fusidic acid

Drug	Solvents	Observation/Inference
Fusidic acid	Water	Soluble
	Methanol	Soluble
	Ethanol	Freely Soluble
	DMSO	Slightly soluble
	Acetone	Soluble
	Chloroform	Soluble
Diclofenac	Water	Insoluble
	Ethanol	Soluble
	Methanol	Freely soluble
	Chloroform	Slightly soluble with heat
	PBSbuffer7.2	Insoluble
	DMSO	Freely soluble
	Ethyl ether	Slightly soluble
	Ethyl acetate	Slightly soluble
	Hexane	Soluble
	Acetone	Soluble

The solubility of Fusidic acid and Diclofenac was determined in various non-volatile or volatile liquid vehicles such as water, methanol, ethanol, DMSO, acetone and chloroform shown in Table 2.

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Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article received- Revised- Accepted-

3.1.3 Determination of melting point

Table 3: Melting Point

S. No.	Drug	Specification	Inference
1.	Fusidic acid	191°C~194°C	192°C±0.021
2.	Diclofenac	155-159°C	157°C

The capillary method was used to determine the melting point of a substance. The melting point of the Fusidic acid was found to be 192^{0}C, which was well within the limits of the drug specification range. The melting point of Diclofenac shows the initial temperature 152°C at which the melting properly started and at 157°C compound melted.

3.1.4 Determination of pH

Table 4: pH of the Fusidic acid

S. No.	Drug	Specification	Inference
1	Fusidic acid	7-8	7.4±0.049

The digital pH meter used to determination the pH of the Fusidic acid. These were found to be 7.4±0.049. These were well within the limits of the drug specification range.

3.1.5 Determination of Partition coefficient

Table 5: Partition coefficient of the Fusidic acid

S. No.	Drug	Inference
1	Fusidic acid	5.25±0.011
2.	Diclofenac	4.98

The Partition coefficient of the Fusidic acid was 5.25±0.011 through this it was observed that the compound was hydrophilic in nature. The partition co efficient of the Diclofenac was 4.98 (K). This result reveals that it was lipophilic in nature.

3.1.6 Lambda max of Fusidic acid and Diclofenac

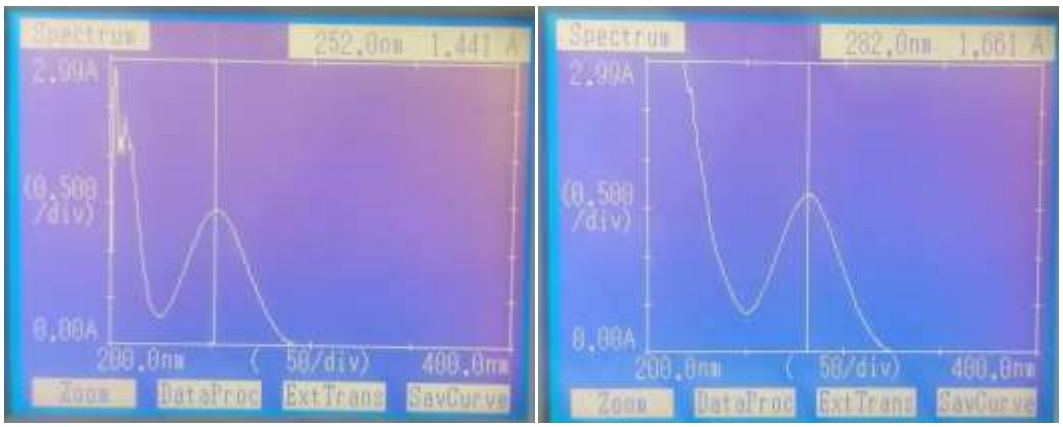

Figure1: Lambda max of Fusidic acid and Diclofenac

Table 6: Lambda max

Sr. No	Drug	UV absorption maxima (Lambda max)
1.	Fusidic acid	252.0nm
2.	Diclofenac	282.0nm

UV- visible spectrophotometer (1700- Shimadzu) was used to determine the lambda max (absorption maxima) of a substance. The lambda max of the Fusidic acid and Diclofenac was found to be 252 nm and 282 nm respectively. This was well within the limits of the drug specification. The difference in the wavelength was admissible because it was permissible ±5 range.

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3.1.7 Standard curve of Fusidic acid

Table 7: Calibration curve of Fusidic acid and Diclofenac

Concentration(µg/ml)	Fusidic acid	Concentration(µg/ml)	Diclofenac
10	0.092	5	0.072
20	0.208	10	0.158
30	0.326	15	0.241
40	0.439	20	0.338
50	0.529	25	0.432
60	0.646	30	0.514
Mean	0.373333	**Mean**	0.2925
SD	0.205726	**SD**	0.167305
%RSD	55	**%RSD**	57

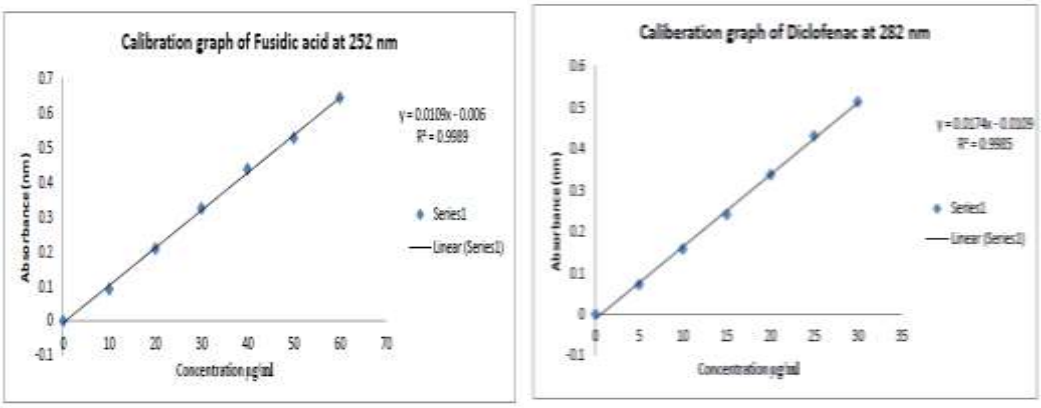

Figure2: Calibration curve of Fusidic acid at 252nm and Diclofenac at 282nm

The linearity of the proposed method was established by least squares linear regression analysis of the calibration curve. The regression equation of Fusidic acid and Diclofenac was obtained by plotting absorbance versus concentration of Fusidic acid and Diclofenac in the range of 10-60 µg/mL and 5-30 µg/ml respectively. Six points calibration curve were obtained in a concentration range from 10-60 µg/mL for drug. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.010x - 0.006 for Fusidic acid and y = 0.017x - 0.010 for Diclofenac with correlation coefficient $R^2 = 0.998$.

3.1.8 Precision study

Table 8: Precision of Fusidic acid

Concentration (µg/ml)	Absorbance	Statistical analysis
20	0.208	
20	0.211	**Mean**
20	0.214	
20	0.222	**SD**
20	0.232	
20	0.219	**%RSD**

Table 9: Precision of Diclofenac

Concentration(µg/ml)	Diclofenac
15	0.239
15	0.241
15	0.238
15	0.236
15	0.235
15	0.234
Mean	0.237167
SD	0.002639
%RSD	1.1129

a) Intraday Precision

Table 10: Intraday Precision of Fusidic acid

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3
20	0.21	0.218	0.207
20	0.217	0.215	0.215
20	0.211	0.214	0.214
20	0.212	0.21	0.218
20	0.217	0.219	0.213
20	0.219	0.22	0.217
Mean	0.214333	0.216	0.214
SD	0.003777	0.003742	0.003899
%RSD	0.017623	0.017322	0.018218
AVG%RSD	1.7721		

Table11: Intraday Precision of Diclofenac

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3
15	0.243	0.233	0.245
15	0.234	0.245	0.239
15	0.235	0.24	0.238
15	0.237	0.236	0.237
15	0.238	0.239	0.244
15	0.235	0.238	0.246
Mean	0.237	0.2385	0.2415
SD	0.003286	0.004037	0.003937
%RSD	1.3866	1.6928	1.6302
AVG%RSD	1.5		

b) Inter day precision

Table12: Inter-day Precision of Fusidic acid

Concentration (µg/ml)	Absorbance (Day 1)	Absorbance (Day 2)	Absorbance (Day 3)
20	0.207	0.201	0.206
20	0.217	0.205	0.208
20	0.210	0.210	0.207
20	0.209	0.211	0.205
20	0.211	0.214	0.212
20	0.206	0.204	0.203
Mean	0.21	0.2075	0.206833
SD	0.003899	0.00493	0.003061
%RSD	1.8565	2.3757	1.4797
AVG%RSD	1.904		

Table 13: Inter-day Precision of Diclofenac

Concentration (µg/ml)	Absorbance (Day 1)	Absorbance (Day 2)	Absorbance (Day 3)
15	0.24	0.239	0.241
15	0.236	0.24	0.243
15	0.234	0.242	0.237
15	0.241	0.236	0.238
15	0.242	0.237	0.243
15	0.245	0.246	0.245
Mean	0.239667	0.24	0.241167
SD	0.004033	0.003317	0.003125
%RSD	1.6828	1.3819	1.2959
AVG%RSD	1.3		

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). The intermediate precision was assessed by comparing the results obtained on three different days. The experimental values (%RSD) obtained for repeatability (intra-day precision) and intermediate precision (inter-day precision) were found to be1.7and 1.9 for Fusidic acid, 1.5 and 1.3 for Diclofenac respectively. If the value not exceeded the average %RSD more than 2 %that means the method was suitable for the drug.

3.2 Ruggedness

Table14: Result of ruggedness of Fusidic acid

Analyst-1		**Analyst-2**	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
20	0.209	20	0.211
20	0.207	20	0.21
20	0.22	20	0.211
20	0.211	20	0.215
20	0.207	20	0.216
20	0.215	20	0.209
Mean	0.2115	**Mean**	0.212
SD	0.005128	**SD**	0.002828
%RSD	2.4248	**%RSD**	1.3342

Table 15: Result of ruggedness of Diclofenac

Analyst-1		**Analyst-2**	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
15	0.244	15	0.237
15	0.245	15	0.247
15	0.246	15	0.248
15	0.239	15	0.25
15	0.243	15	0.241
15	0.242	15	0.243
Mean	0.243	**Mean**	0.244
SD	0.002483	**SD**	0.004885
%RSD	1.0212	**%RSD**	1.9995

Ruggedness of the proposed method was evaluated by comparison of the absorbance of Fusidic acid and Diclofenac that have been measured by two different analysts in the same laboratory. Ruggedness was carried out at 20 µg/ml and **15** µg/ml concentration respectively. The results are expressed as standard deviation and relative standard deviation and it was found 0.211±0.005128 for analyst-1and 0.212±0.002828 for analyst-2 whereas the value of % RSD was recorded 2.4 and1.3 respectively for Fusidic acid. The results were expressed as standard deviation and relative standard deviation and it was found 0.243±0.002483 for analyst-1 and 0.244 ± 0.004885 for analyst-2 whereas the value of % RSD was recorded 1.02 and 1.9 respectively for Diclofenac. This result reveals that the analyst I RSD % was equivalent to 2% but it was permissible and Analyst II % result was up to the mark.

3.3 Robustness

Table16: Results showing robustness of Fusidic acid

Temperature25^{0}C		**Temp30^{0}C**	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
20	0.204	20	0.208
20	0.21	20	0.216
20	0.204	20	0.215
20	0.203	20	0.209
20	0.211	20	0.199
20	0.212	20	0.198
Mean	**0.207333**	**Mean**	**0.196667**
SD	**0.004082**	**SD**	**0.02383**
%RSD	**1.969**	**%RSD**	**1.21169**

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http://www.veterinaria.org
Article received- Revised- Accepted-

Table 17: Robustness of Diclofenac

Temperature25^{0}C		**Temp30^{0}C**	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
15	0.238	15	0.234
15	0.237	15	0.232
15	0.236	15	0.236
15	0.233	15	0.237
15	0.229	15	0.229
15	0.241	15	0.228
Mean	0.235	Mean	0.232
SD	0.004179	SD	0.00367
%RSD	1.7	%RSD	1.5

The robustness of the proposed method was assessed with changes in the analytical temperature. Robustness was carried out at concentration 20 µg/ml at temperature 25^{0}C and 30^{0}C. The results are expressed as standard deviation and relative standard deviation and were recorded 0.207± 0.004082 and 0.196± 0.02383 whereas % RSD was found 1.96 and 1.21 respectively.

3.4 SIMULTANEOUS ESTIMATION

Determination of Isosbestic point and selection of suitable Wavelength

An Isosbestic λ point (a wavelength of equal absorptivity of the two components) was determined by taking overlain spectrum of the solutions Fusidic acid and **Diclofenac** in methanol in UV range against the solvent blank. From the overlain spectra of the two drugs, it was found that Fusidic acid showed λ max at 252 nm and Diclofenac showed λ max at 282 nm. Iso-absorptive point was found out at 265 nm, as Iso-absorptive point was selected for estimation of Drug simultaneously shows in fig: 3

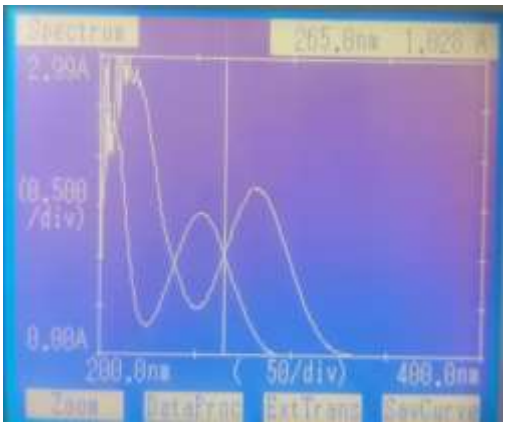

Figure 3: Overlay spectra of Fusidic acid and diclofenac with Iso-absorptive point

The individual concentration range for Beer-Lambert was found 10-60 and 5-30 µg/ml for both Fusidic acid and Diclofenac at 252nmand 282 nm with correlation coefficient 0.998 and 0.998. UV scan of solution of both the combination of drug showed the absorption maxima at 252 nm, 282 nm and265 nm (isosbestic point). The simultaneous estimation was done to check the interference between both the drugs at the λ max of one another. By substituting absorbance and absorptivity values in simultaneous equation, C1 and C2 were calculated, Cx: 4.49 µg/ml, C2: 13.10 µg/ml. The percentage of Fusidic acid and Diclofenac recovered after the combination was found to be 78.9 % and 88.6 % respectively indicating no interference between both the drugs. The Linearity was observed by the linear regression equation method for Fusidic acid and Diclofenac indifferent concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. Hence proposed method can be used for routine analysis of these two drugs in combined dosage form. It was validated as per ICH guidelines.

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3.5 FTIR study of the Fusidic acid Compatibility study by FTIR

1. Fusidic acid

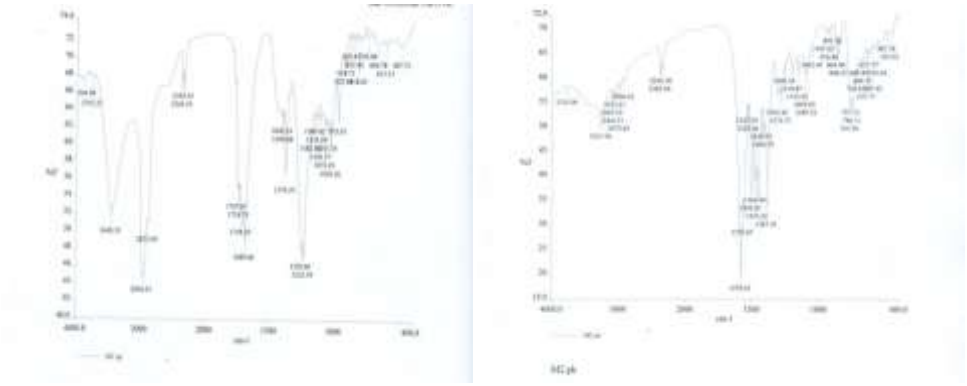

Figure 4: FTIR graph of Fusidic acid and Diclofenac

2) Fusidic acid+ Diclofenac

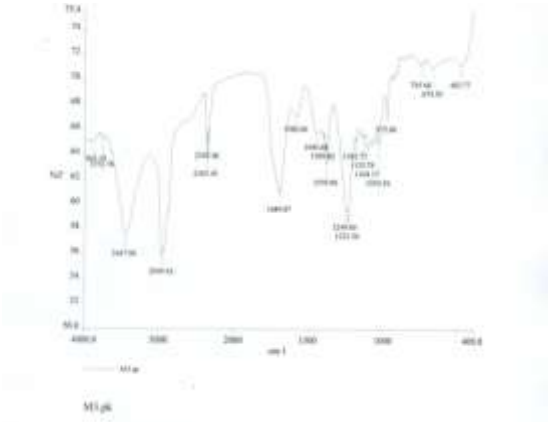

Figure 5: FTIR graph of Fusidic acid+ Diclofenac

Table 18: FTIR Interpretation

Sr. No.	Drugs	Frequency Range	Group Absorption (cm-1)	Appearance	Group	Compound Class
1	Fusidic acid (M1)	3550-3200 (cm-1)	3448.10	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2840 (cm-1)	2950.91	Medium	C-H stretching	Alkane
		3000-2840 (cm-1)	2873.09	Medium	C-H stretching	Alkane
		2400-2000 (cm-1)	2365.55	Strong	C-H stretching	Alkane
		2350-2340 (cm-1)	2345.55	Strong	O=C=O stretching	Carbondioxide
		1750-1735 (cm-1)	1747.67	Strong	C=O stretching	Ester
		1740-1720 (cm-1)	1734.75	Strong	C=O stretching	Aldehyde
		1720-1706 (cm-1)	1718.19	Strong	C=O stretching	Carboxylic acid
		2000-1650 (cm-1)	1689.06	Medium	C-H bending	Aromatic compound

		1420-1330 (cm-1)	1406.14	Medium	O-H bending	Alcohol
		1440-1395 (cm-1)	1399.88	Medium	O-H bending	Carboxylic acid
		1420-1330 (cm-1)	1376.55	Medium	O-H bending	Alcohol
		1275-1200 (cm-1)	1233.59	Strong	C-O stretching	Alkylaryl ether
		1210-1163 (cm-1)	1182.82	Strong	C-O stretching	Ester
		1400-1100 (cm-1)	1106.57	Weak	C-C stretching	Alkane
		1085-1050 (cm-1)	1073.91	Strong	C-O stretching	Alcohol
		1085-1050 (cm-1)	1053.35	Strong	C-O stretching	Alcohol
		985-960 (cm-1)	975.87	Strong	C=C stretching	Alkene
		840-790 (cm-1)	818.65	Medium	C=C bending	Substitute
		760-740 (cm-1)	750.98	Strong	C-H bending	Substituted
2	**Diclofenac (M2)**	3300-2500 (cm-1)	3247.08	Strong, Broad	O-H stretching	Carboxylic acid
		3100-2800 (cm-1)	3066.51	Strong, Broad	N-H stretching	Amine
		3100-3000 (cm-1)	3035.61	Medium	C-H stretching	Alkene
		3000-2840 (cm-1)	2906.42	Medium	C-H stretching	Alkane
		2400-2000 (cm-1)	2365.46	Strong	C-H stretching	Alkane
		2350-2340 (cm-1)	2345.50	Strong	O=C=O stretching	Carbondioxide
		1650-1566 (cm-1)	1578.03	Medium	C=C stretching	Alkene
		1600-1400 (cm-1)	1498.84	Strong	C=C stretching	BenzeneRing
		1480-1450 (cm-1)	1466.49	Medium	C-H bending	Alkene
		1460-1450 (cm-1)	1450.42	Medium	C-H bending	Alkane
		1440-1395 (cm-1)	1406.39	Medium	O-H bending	Carboxylic acid
		1420-1330 (cm-1)	1381.93	Medium	O-H bending	Alcohol
		1310-1250 (cm-1)	1274.71	Strong	C-O stretching	Aromaticester

		1210-1163 (cm-1)	1194.87	Strong	C-O stretching	Ester
		1400-1100 (cm-1)	1152.65	Weak	C-C stretching	Alkane
		1085-1050 (cm-1)	1099.99	Strong	C-O stretching	Alcohol
		1085-1050 (cm-1)	1089.22	Strong	C-O stretching	Alcohol
		1250-1020 (cm-1)	1042.40	Medium	C-N stretching	Amine
		960-920 (cm-1)	947.05	Strong	C=C bending	Alkene
		850-550 (cm-1)	846.27	Strong	C-Cl stretching	Halo compound
		840-760 (cm-1)	765.90	Medium	C=C bending	Trisubstitute
		730-665	716.45	Strong	C=C	Disubstitute
		(cm-1)			bending	
		730-665 (cm-1)	669.75	Strong	C=C bending	Disubstitute
		850-550 (cm-1)	837.77	Strong	C-Cl stretching	Halo compound
		850-550 (cm-1)	587.45	Strong	C-Cl stretching	Halo compound
		850-550 (cm-1)	553.64	Strong	C-Cl stretching	Halo compound
3	**s Fusi+Diclo (M3)**	3550-3200 (cm-1)	3447.96	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2840 (cm-1)	2949.42	Medium	C-H stretching	Alkane
		2400-2000 (cm-1)	2365.43	Strong	C-H stretching	Alkane
		2350-2340 (cm-1)	2345.46	Strong	O=C=O stretching	Carbondioxide
		2000-1650 (cm-1)	1689.87	Medium	C-H bending	Aromatic compound
		1650-1566 (cm-1)	1580.06	Medium	C=C stretching	Alkene
		1600-1400 (cm-1)	1444.68	Strong	C=C stretching	BenzeneRing
		1440-1395 (cm-1)	1399.82	Medium	O-H bending	Carboxylic acid
		1420-1330 (cm-1)	1379.90	Medium	O-H bending	Alcohol
		1310-1250 (cm-1)	1249.40	Strong	C-O stretching	Aromaticester
		1275-1200 (cm-1)	1232.50	Strong	C-O stretching	Alkylaryl ether

		1210-1163 (cm-1)	1182.77	Strong	C-O stretching	Ester
		1400-1100 (cm-1)	1133.74	Weak	C-C stretching	Alkane
		1400-1100 (cm-1)	1104.17	Weak	C-C stretching	Alkane
		1250-1020 (cm-1)	1030.36	Medium	C-N stretching	Amine
		985-960 (cm-1)	975.86	Strong	C=C stretching	Alkene
		760-740 (cm-1)	745.66	Strong	C-H bending	Substituted
		850-550 (cm-1)	670.50	Strong	C-Cl stretching	Halo compound

3.6 HPLC GRAPH

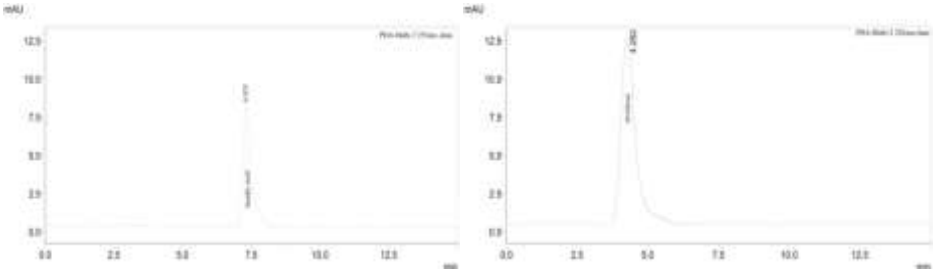

Figure 6: HPLC graph of the Fusidic acid at channel 252 nm and at 282 nm

4. CONCLUSION

In conclusion, the preformulation studies and simultaneous estimation of Fusidic Acid and Diclofenac presented in this research provide a comprehensive understanding of the physicochemical properties, compatibility, and analytical profiles of these drugs. The findings underscore the importance of preformulation studies in the development of effective and stable pharmaceutical formulations. The successful simultaneous estimation of Fusidic Acid and Diclofenac further demonstrates the viability of the analytical methods employed, offering a reliable approach for quality control in combined drug formulations. This study lays a solid foundation for the development of dual-drug therapies, potentially enhancing therapeutic outcomes while ensuring the safety and efficacy of the formulations. Future research could build upon these findings to explore novel delivery systems and optimize formulations for clinical use.

5. REFERENCES

1. Almostafa, M. M., Elsewedy, H. S., Shehata, T. M., & Soliman, W. E. (2022). Novel formulation of fusidic acid incorporated into a myrrh-oil-based nanoemulgel for the enhancement of skin bacterial infection treatment. Gels, 8(4), 245.
2. Biedenbach, D. J., Rhomberg, P. R., Mendes, R. E., & Jones, R. N. (2010). Spectrum of activity, mutation rates, synergistic interactions, and the effects of pH and serum proteins for fusidic acid (CEM-102). Diagnostic microbiology and infectious disease, 66(3), 301-307.
3. Brahmankar, D. M., & Jaiswal, S. B. (2019). Biopharmaceutics and pharmacokinetics. Vallabh prakashan.
4. Chatwal, G. R. (2022). Instrumental methods of chemical analysis. Himalaya publishing house.
5. Corey, G. R. (2009). Staphylococcus aureus bloodstream infections: definitions and treatment. Clinical Infectious Diseases, 48(Supplement_4), S254-S259.
6. Djordjevic, L., Primorac, M., & Stupar, M. (2005). In vitro release of diclofenac diethylamine from caprylocaproyl macrogolglycerides based microemulsions. International journal of pharmaceutics, 296(1-2), 73-79.
7. Dong, N. T., Trang, N. T. T., Hoang, N. V., Linh, P. T., & Yen, P. T. (2023). Simultaneous determination of paracetamol and diclofenac in wastewater by high-performance liquid chromatography method. Vietnam Journal of Science and Technology, 599-608.
8. Godtfredsen, W. O., Jahnsen, S., Lorck, H., Roholt, K., & Tybring, L. (1962). Fusidic acid: a new antibiotic. Nature, 193(4819).
9. Gondalia, R., Mashru, R., & Savaliya, P. (2010). Development and validation of spectrophotometric methods for simultaneous estimation of ibuprofen and paracetamol in soft gelatin capsule by simultaneous equation

method. International Journal of ChemTech Research, 2(4), 1881-1885.
10. Gopinath, R., & Naidu, R. A. S. (2011). Pharmaceutical preformulation studies–current review. International Journal of Pharmaceutical and Biological Archives, 2(5), 1391-1400.
11. Jyoti, K., Malik, G., Chaudhary, M., Sharma, M., Goswami, M., Katare, O. P., ... & Madan, J. (2020). Chitosan and phospholipid assisted topical fusidic acid drug delivery in burn wound: Strategies to conquer pharmaceutical and clinical challenges, opportunities and future panorama. International journal of biological macromolecules, 161, 325-335.
12. Khichariyaa, A., Jeswania, G., Vermaa, V. S., Sahua, S., Rathora, S., Alexander, A., & Nakhate, K. T. (2022). Estimation of Diclofenac diethylamine and Plumbagin in a drug delivery system by simultaneous equation method. NeuroQuantology, 20(8), 5013.
13. Lachman, L., Lieberman, H. A., & Kanig, J. L. (1976). The theory and practice of industrial pharmacy (pp. 210-212). Philadelphia: Lea & Febiger.
14. Lau, E. (2001). Preformulation studies. In Separation science and technology (Vol. 3, pp. 173-233). Academic Press.
15. Lode, H. M. (2009). Clinical impact of antibiotic-resistant Gram-positive pathogens. Clinical microbiology and infection, 15(3), 212-217.
16. Patel, P. (2019). Preformulation studies: an integral part of formulation design. Pharmaceutical Formulation Design-Recent Practices, 9.
17. Shah, P., Pandya, T., Gohel, M., & Thakkar, V. (2019). Development and validation of HPLC method for simultaneous estimation of rifampicin and ofloxacin using experimental design. Journal of Taibah University for science, 13(1), 146-154.
18. Verma, G., & Mishra, M. K. (2016). Pharmaceutical preformulation studies in formulation and development of new dosage form: A review. Int. J. Pharma Res. Rev, 5(10).
19. Vilegave, K., Vidyasagar, G., & Chandankar, P. (2013). Preformulation studies of pharmaceutical new drug molecule and products: An Overview. The American Journal of Pharmacy, 1(3), 1-20.
20. Wadhwa, S., Singh, B., Sharma, G., Raza, K., & Katare, O. P. (2016). Liposomal fusidic acid as a potential delivery system: a new paradigm in the treatment of chronic plaque psoriasis. Drug delivery, 23(4), 1204-1213.
21. Yuan, X., Liu, R., Zhang, W., Song, X., Xu, L., Zhao, Y., ... & Zhang, J. (2021). Preparation of carboxylmethylchitosan and alginate blend membrane for diffusion-controlled release of diclofenac diethylamine. Journal of Materials Science & Technology, 63, 210-215.