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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD OF DICLOFENAC SODIUM AND FAMOTIDINE BY Q-ABSORPTION METHOD

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ABSTRACT

The Research provides a Q-absorbance ratio method for determining diclofenac sodium and famotidine in their combined pharmaceutical dosage form that is simple, sensitive, quick, accurate, precise, and cost-effective. The absorbance ratio method compares absorbance at two wavelengths, one of which is an iso-absorptive point and the other which is the λ-max of one of the two components. In methanol, the iso-absorptive point for diclofenac sodium and famotidine is 279.20 nm. The second wavelength is 282 nm, which is diclofenac sodium's λ-max in methanol. Linearity was determined in the concentration ranges of 10-20 µg/ml for diclofenac sodium and 2-12 µg/ml for famotidine. The ratio of absorbance at the iso-absorptive point and at the λ-max of diclofenac sodium was used to calculate the drug concentration. Because there was no interference, the method was effectively applied to tablet dosage form. Recovery investigations have confirmed the findings of the research.

Keywords: Iso-absorptive point, Diclofenac Sodium, Famotidine

1. INTRODUCTION

Famotidine3-[({2-[(diaminomethylidene) amino]-1,3-thiazol-4-yl}methyl)sufanyl]-N-sulfamoyl propan -imidamide. It blocks the H2 receptor. It prevents histamine from acting on parietal cells, thus lowering stomach acid output **[1, 3]**. Diclofenac Sodium [2-(2,6-Dichloroanilino) phenyl] acetic acidis a non-steroidal anti-inflammatory. It is anti-inflammatory, antipyretic, and analgesic effects are considered to be caused by COX inhibition, which prevents prostaglandin formation. Inhibiting COX-1 and COX-2 with comparatively equal strength is diclofenac **[2, 4]**.

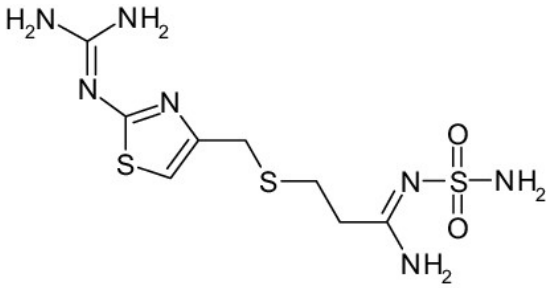

Figure 1: Structure of Famotidine

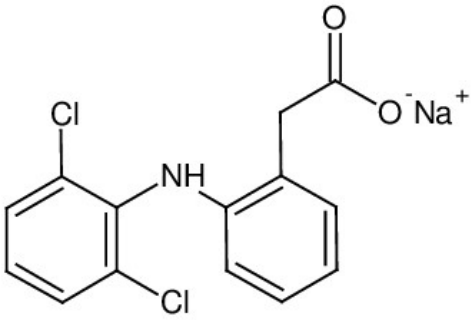

Figure 2: Structure of Diclofenac Sodium

Several techniques (UV, HPLC, and HPTLC) were published from literature review for the analysis of individual drugs and combinations of drugs, but no methods were reported for the simultaneous estimate of famotidine and diclofenac sodium using Q-absorption Ratio method. The goal of the current investigation isto determine Q-absorbance ratio spectrophotometric approach for the simultaneous measurement of famotidine and diclofenac sodium in combination dosage form.

2. MATERIALS AND METHODS:

2.1 Apparatus

UV Spectrophotometric measurements were made Shimadzu UV – visible double beam spectrophotometer (Model- 1800), Electronic balance Shimadzu BL-220H

with capacity 220g and readability of 0.001g, Infrared spectroscopy Bruker Alpha 2 platinum ATR.

2.1.2 Reagents and solutions

Famotidine procured as gift sample from N Cube pharmaceutical Pvt. Ltd. Bavla, Ahmedabad, Diclofenac sodium procured as gift sample from Intas pharmaceutical Ltd. Ahmedabad, Combined dosage form of Famotidine 20 mg and Diclofenac Sodium 75 mg, Fenlog Tablet Procured from Market (Mfg. By SKN Organics PTV. LTD.), Methanol (Analytical grade) was purchased from S.D. Fine Chemicals.

2.1.3 Preparation of standard stock solution

To prepare a stock solution with a concentration of 100 µg/ml, a precisely weighed quantity of famotidine (10 mg) was transferred to a 100 ml volumetric flask add 10 ml of methanol, and make up the volume up to the mark using methanol. To prepare a stock solution with a concentration of 100 µg/ml, a precisely weighed quantity of Diclofenac sodium (10 mg) was transferred to a 100 ml volumetric flask add 10 ml of methanol, and make up the volume up to the mark using methanol

2.1.4 Preparation of sample solution

From thestandard stock solution prepared to get 2, 4, 6, 8, 10 and 12 µg/ml of Femotidine and 10, 12, 14, 16, 20 µg/ml for Diclofenac sodium respectively.

2.1.5 Method Validation

In terms of linearity, range, repeatability, method precision, intermediate precision, accuracy, LOD, LOQ, robustness, ruggedness, and system adaptability, the suggested method was verified.

2.1.6 Linearity and range

The linearity curve was determined by graphing concentration vs. absorbance. Calibration curves were prepared using 2, 4, 6, 8, 10 and 12 µg/ml of Femotidine and 10, 12, 14, 16, 20 µg/ml for Diclofenac sodium respectively. The findings are summarised in a **Table 1 and 2**. **[10, 11]**.

2.1.7 Precision:

The precision of an analytical method is determined by using six homogeneous sample concentration of 8 µg/ml under normal operating conditions **[5]**. The percentrelative standard deviation (% RSD) was calculated.

2.1.8 Accuracy:

The accuracy is the closeness of agreement between test and the true value. Accuracy is the percentage of analyte recovered by assay from known added amount **[6]**. At 80%, 100%, and 120% levels of standard solution, the analytical method's accuracy for diclofenac and famotidine was measured. Nine determinations were made using three concentration levels with three replicates each, yielding data that covered the necessary range. Results of the absorbance measurement at 276.20 nm were represented as a percentage of

recoveries. RSD percentage and standard deviation were computed.

2.1.9 Ruggedness:

Ruggedness of the method was confirmed by analysis of samples under a changing of conditions such as change in instrument and analyst and effect on the % assay was studied **[7]**.

2.1.10 Robustness:

Ruggedness of the method was confirmed by analysis of samples under a changing of conditions such as change in wavelength and effect on the %Assay was studied **[7]**.

2.1.11 Limit of detection and limit of quantification

Calculating the signal-to-noise ratio (i.e. 3.3 for LOD and 10 for LOQ) using the following formulae indicated by the International Conference on Harmonization (ICH) guideline yielded the drug's limit of detection (LOD) and limit of quantification (LOQ):

$$\mathbf{LOD = 3.3 \text{ and } LOQ = 10 \text{ X } \sigma/S}$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

3. RESULTS & DISCUSSION

A simple, selective, accurate, precise spectrophotometric method for the estimation of Famotidine and Diclofenac in bulk and pharmaceutical tablet dosage form has been developed and validated. The UV spectrum of diluted solutions for various concentrations of diclofenac sodium and Famotidine in the methanol was prepared and recorded absorbance using UV spectrophotometer. λmax for famotidine was found to be 288nm and λmax for Diclofenac Sodium was found to be 282nm. Famotidine's response was linear in the concentration range of 2–12 µg/ml and diclofenac sodium response was linear in the concentration range of 10-20 µg/ml. Famotidine and Diclofenac Sodium were found to have correlation coefficients of R = 0.99839 and 0.99287 respectively **(Table 1, 2 and Figure 3 and 4)**. The iso-absorptive point that crossed over during the overlay of famotidine and diclofenac sodium was found at 279.2 nm **(Figure 9)**. For famotidine and diclofenac sodium, the mean% recoveries were found between 98.00 and 98.51% respectively **(Table 7)**. The LOD and LOQ were 1.715 µg/ml and 5.198 µg/ml of Famotidine 1.42 µg/ml and 4.31 µg/ml of Diclofenac sodium, respectively **(Table 6)**. The suggested method was accurate, precise, repeatable, and had an acceptable recovery of the analyte. It can be used to analyse pharmaceutical formulations containing both Famotidine and Diclofenac Sodium. The ICH guidelines were followed in every step of the current study. **Table 7** shows the results of the method's validation in terms of accuracy, precision, repeatability, LOD, LOQ, ruggedness, and system suitability.

Table 1: Linearity of Famotidine

Sr. no.	Concentration	Absorbance (WL 288.0)
1	0	0
2	2	0.107
3	4	0.200
4	6	0.312
5	8	0.395
6	10	0.517
7	12	0.594

Table 2: Linearity of Diclofenac Sodium

Sr. no.	Concentration	Absorbance WL 282.0
1	0	0
2	10	0.295
3	12	0.350
4	14	0.455
5	16	0.525
6	18	0.590
7	20	0.663

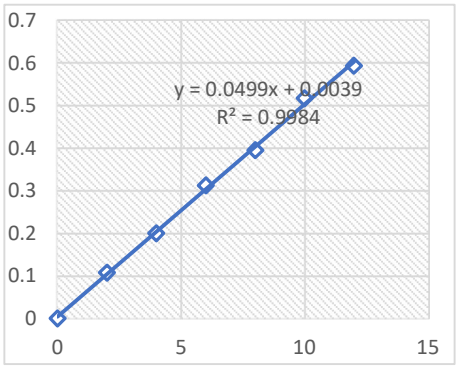

Figure 3: Calibration curve of Famotidine

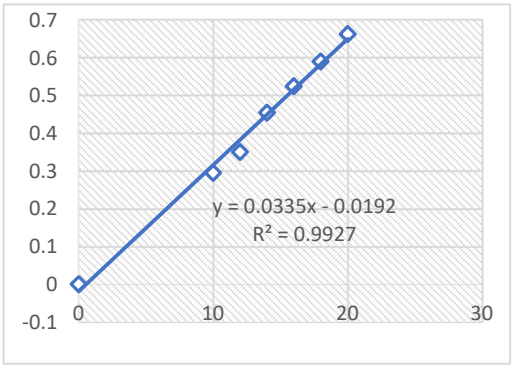

Figure 4: Calibration curve of Diclofenac Sodium

IR SPECTROSCOPY FOR FAMOTIDINE AND DICLOFENAC SODIUM

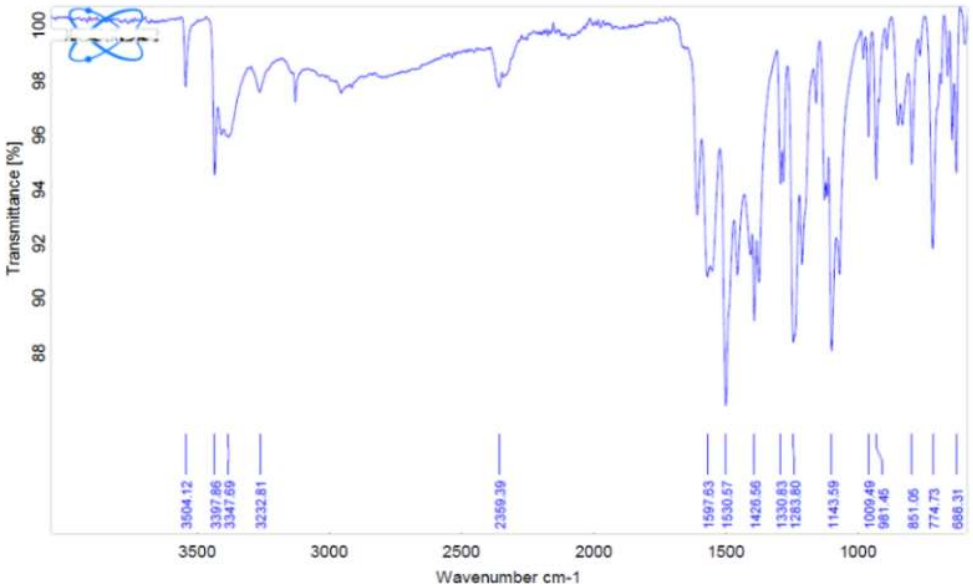

Figure 5: IR of Famotidine

Table 3: IR Interpretation of Famotidine

Absorbance (cm^{-1})	Group	Compound Class
3504	N-H stretching	Asymmetric stretching of Primary amine
3397	N-H stretching	Symmetric stretching of Primary amine
1597, 1530	C=C stretching	Aromatic C=C streching
1428	CH2 bending	Alkane
1143	C-N bending	Amines
1009	S = O	Sulphoxidecompound

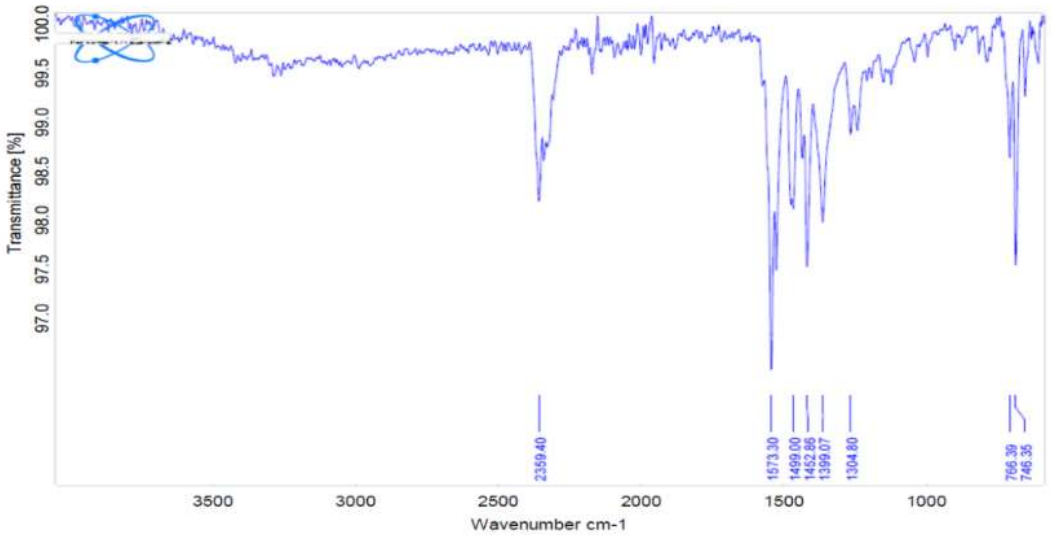

Figure 6: Interpretations for Famotidine

Table 4: IR Interpretation of Diclofenac Sodium

Absorbance (cm^{-1})	Group	Compound Class
2359	Aromatic CH	C-H stretching of Aromatic
1673,,1449	C=C aromatics'	Aromatic
1452	CH2 bending	Alkane
1339	C-C	Alkane
1304	C-O	Ester
766	C-Cl	Chlorine

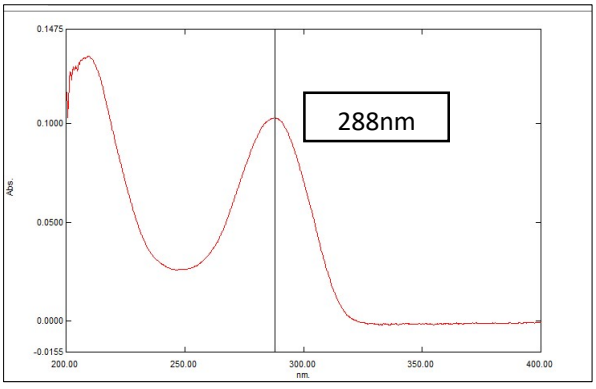

Figure 7: UV Spectra of Famotidine(λmax=288nm)

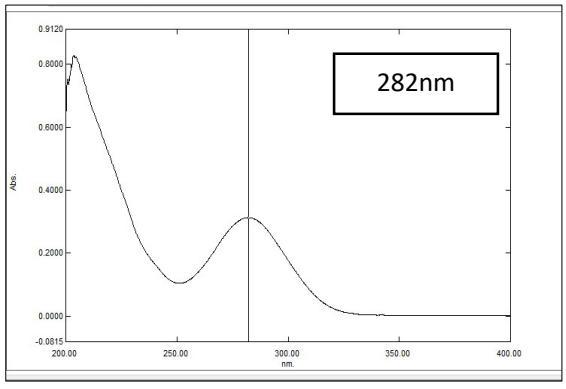

Figure 8: UV Spectra of Diclofenac Sodium(λmax=282nm)

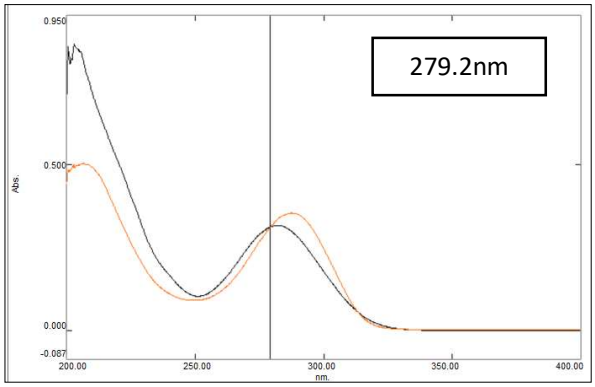

Figure 9: Overlay of Famotidine and Diclofenac Sodium(λmax=279.2nm)

Table 5: Ruggedness and Robustness

Parameter	Assay with Normal condition		Assay with changed condition	
	Result of Famotidine % Assay	Result of Diclofenac Sodium % Assay	Result of Famotidine % Assay	Result of Diclofenac Sodium % Assay
Change in Instrument	98.0%	98.51%	98.21%	98.58%
Analyst Change	98.0%	98.51%	98.16%	98.55%
Wavelength Change	98.0%	98.51%	98.19%	98.48%

Table 6: Summary of the method's proposed validation parameters

Parameter	Result (Famotidine)	Result (Diclofenac Sodium)
Wavelength (nm)	288.0	282.0
Beer's Law Limit(µg/ml)	2-10µg/ml	10-20µg/ml
Regression Equation	Y= 0.0226 x + 0.3509	Y= 0.009 x + 0.3999
Correlation coefficient(r^2)	0.99839	0.99287
Precision (SD) %	0.000271	0.00003807
Precision (RSD)%	0.060479	0.06489
Accuracy:80% 100% 120%	98.64% 98.00% 97.89%	97.30% 98.51% 97.76%
Robustness (Wavelength change)	98.19%	98.48%
Ruggedness: Instrument change Analyst change	98.21% 98.16%	98.58% 98.55%
LOD	1.715	1.42313
LOQ	5.198	4.3125
Overlay of famotidine and diclofenac sodium	279.2nm	

Table 7: Accuracy of Famotidine

Amount added	Day1	Day2	Day3	% Recovery found		
80% 18ppm				Day1	Day2	Day3
1	0.897	0.897	0.888			
2	0.868	0.884	0.887	98.16%	98.72%	99.05%
3	0.899	0.894	0.907			
Mean	0.886	0.891	0.894			
100% 20ppm						
1	0.984	0.985	0.964			
2	0.974	0.969	0.991	98.30%	97.90%	97.80%
3	0.988	0.991	0.987			
Mean	0.985	0.981	0.980			
120% 22ppm						
1	1.035	1.101	1.036			
2	1.085	1.021	1.120	97.47%	97.56%	98.66%
3	1.102	1.103	1.105			
Mean	1.074	1.075	1.084			

Table 8: Accuracy of Diclofenac sodium

Amount added	Day1	Day2	Day3	% Recovery found		
80%18ppm				Day1	Day2	Day3
1	0.596	0.554	0.587			
2	0.549	0.578	0.554	98.54%	95.94%	97.44%
3	0.587	0.555	0.574			
Mean	0.577	0.562	0.571			
100%20ppm						
1	0.618	0.629	0.634			
2	0.623	0.654	0.627	96.04%	99.75%	99.75%
3	0.619	0.636	0.658			
Mean	0.620	0.652	0.639			
120%22ppm						
1	0.721	0.680	0.713			
2	0.705	0.638	0.704	99.13%	96.27%	97.90%
3	0.717	0.716	0.699			
Mean	0.714	0.693	0.705			

4. CONCLUSION

The suggested method for estimating famotidine and diclofenac sodium in bulk and pharmaceutical dosage forms is simple, reproducible, accurate, precise and selective. As a result, it can be successfully used for routine analysis. In that formulation the usual excipients and other additives were used, which did not interfere the analysis of the tablet dosage form. So, it is safe to say that this method is used for pharmaceutical dosage forms that include a combined dosage.

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