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Simultaneous Estimation of Naproxen and Famotidine by Analytical Q Absorption Ratio Method

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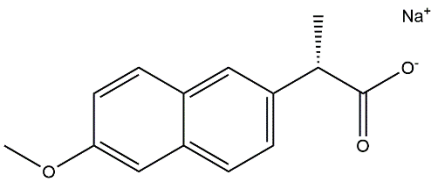
ABSTRACT

A simple, accurate, precise, and repeatable Q-Absorption ratio UV spectroscopic technique was developed for simultaneous estimation of Naproxen and Famotidine. The method was developed according to the International Council of Harmonization guidelines. The solvent used in the analysis was 2% SLS pH (1.4) and the corresponding wavelength selected were 257 and 275nm. Beer's law was followed in the range of 10-60 µg/ml and 5-30 µg/ml for NAP and FAM respectively with regression values of r^2= 0.9934 and 0.9994 at 257 nm 0.9922 and 0.9963 at 275 nm for NAP and FAM respectively. The values of relative standard deviation and % recovery were found to be within the acceptance limit, indicating that the proposed method is precise and accurate and thus can be used for the routine analysis of NAP and FAM in pharmaceuticals.

1. INTRODUCTION-

Naproxen, also chemically known as 2(6'-methoxy-2'-naphthyl)-propanoic acid (figure 1a), is a non-selective cyclooxygenase (COX) inhibitor belonging to the NSAIDs group. Naproxen (NAP) is an FDA-approved drug most commonly used in the treatment of diseases such as polyarticular juvenile idiopathic arthritis, ankylosing spondylitis, bursitis, acute gout, tendonitis, rheumatoid arthritis, pain, osteoarthritis and primary dysmenorrhea(1). NAP is a non-selective COX inhibitor and its long-term administration can cause GI side effects leading to gastric ulcers (2)(3). According to the survey, 15% of patients undergoing treatment with NAP suffer from gastric ulcers (4). Thus, NAP is generally coadministered with H_2 blockers to prevent NSAID-related GI complications. (5)(6). Famotidine (FAM) is a type-2 histaminic receptor antagonist used in the treatment of gastrointestinal ulcers and heartburns and is chemically known as 3[[2-(diamino methylidene amino)-1,3-thiazol-4-yl]methylsulfanyl]N-sulfamoylpropanimidamide (figure 1b).

Famotidine acts by inhibiting excess gastric acid secretion caused by the administration of Naproxen(7)(8). A fixed-dose combination of NAP as NSAID and FAM as H_2 blocker can be formulated for effective treatment of inflammatory disorders such as polyarticular juvenile idiopathic arthritis, ankylosing spondylitis, and bursitis, acute gout, tendonitis, rheumatoid arthritis, pain, osteoarthritis and primary dysmenorrhea with minimized gastric related side effects. Validation methods including UV and HPLC are reported in the literature for the estimation of NAP and FAM individually in pharmaceutical products(9)(10)(11)(12). A comprehensive review of the literature revealed that there is no methodology for simultaneous estimation of NAP and FAM in pharmaceutical preparations. Therefore, a UV spectroscopic-based analytical method for the simultaneous analysis of NAP and FAM is required for their quantitative estimation in the formulation. The goal of the current study was to develop a simple, accurate, and precise absorbance ratio method for the simultaneous determination of NAP and FAM in a pharmaceutical product. Method validation was according to ICH Q2 (R1) protocol(13).



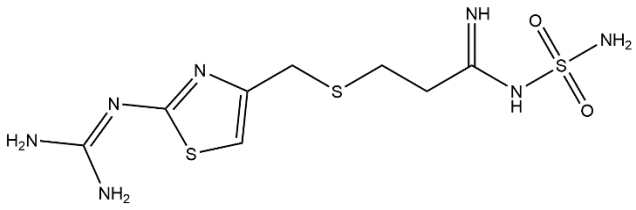


Figure 1a. Naproxen sodium and **Figure 1b. Famotidine**

MATERIALS AND METHODS

Naproxen sodium was purchased from Dhamtec Pharma and Consultants, Mumbai, Maharashtra, India. Famotidine was obtained as a gift sample from Lupin limited (Research Park) Pune, Maharashtra, India. All other chemicals used in the experiment were of analytical grade which were purchased from SD fine. For all investigations, a Shimadzu model 1900 double beam UV-visible spectrophotometer with software UV probe 2.70 and a 1cm quartz cell were used. The weighing of drugs was done on Shimadzu's electrical balance. Dissolution of drugs was done using Dolphin Ultrasonicator. The glassware used in the study was calibrated prior study.

Application of the proposed method for estimation in standard laboratory mixture- Absorptivity coefficients for NAP and FAM were determined and their concentrations in the mixture were determined. The following equation was used to calculate the concentrations of NAP and FAM in a standard laboratory mixture using the established approach.

$$C_{Nap} = \frac{Q_M - Q_Y}{Q_x - Q_y} \times \frac{A_1}{a_{y1}}$$

(1)

$$C_{FAM} = \frac{Q_M - Q_x}{Q_y - Q_x} \times \frac{A_1}{a_{y1}}$$

where $Q_M = A_2/A_1$, $Q_X = a_{X2}/a_{X1}$, and $Q_Y = a_{Y2}/a_{Y1}$; A_1 and A_2 are the absorbances, of the mixture at 257 nm and 275 nm, respectively; a_{X1} and a_{Y1} are absorptivities of Naproxen sodium and Famotidine at 257nm respectively; a_{X2} and a_{Y2} are absorptivities of Naproxen sodium and Famotidine at 275 nm respectively.

Determination of Isoabsorptive wavelength and absorbance maxima (λ_{max})-

Solutions of 40 μg/mL concentrations of both the drugs were prepared by transferring 4 ml of working stock solutions (100 μg/mL) to 10 ml volumetric stock and diluted up to 10 ml with solvent and scanned between 200-400 nm against solvent as blank. Overlay spectra of both the drugs were obtained to determine the absorptive wavelength and the absorbance maxima of NAP and FAM (Figure.2).

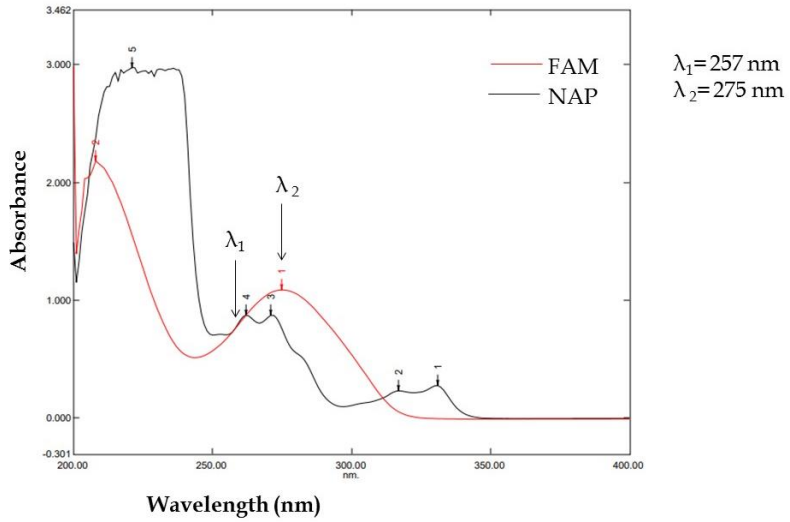


Fig.2 Overlay spectra of NAP and FAM

Preparation of standard stock solutions –

Reference solution:

The standard stock solution of FAM was made by dissolving 100 mg of FAM in 100 mL of solvent and stirring it occasionally to achieve a concentration of 1000 µg/mL. To obtain a 100 µg/mL working standard, 1mL of this solution was diluted to 10 mL using a solvent. Similarly, a stock solution of NAP was made and appropriately diluted to yield a working standard solution of 100µg/mL.

Sample solution

To obtain sample solutions with varying concentrations, aliquots of required quantities were taken from working standard solutions and adequately diluted with solvent.

A calibration curve (Linearity and range)-

The developed method followed Beer's law within a range of 10-60 μg/mL and 5-30 μg/mL for NAP and FAM respectively. Different aliquots of 1, 2, 3, 4, 5, and 6 ml from the NAP working standard solution were transferred and suitably diluted with a solvent to obtain concentrations of 10, 20, 30,40,50, and 60 μg/mL. Different aliquots of 0.5, 1, 1.5, 2, 2.5, and 3 mL from FAM working standard solution were transferred and suitably diluted to obtain concentrations of 5, 10, 15, 20, 25, and 30μg/mL. The absorbance of diluted solutions was determined at the absorptive wavelength (257nm) and λ_{max} of FAM (275 nm). A calibration curve was obtained by plotting absorbance vs concentration (μg/mL). The experiment was performed in triplicates. Linearity curves for NAP and FAM at 257 and 275 nm are depicted in fig 3a and fig 3b. The results of the linearity study are depicted in Table 1.

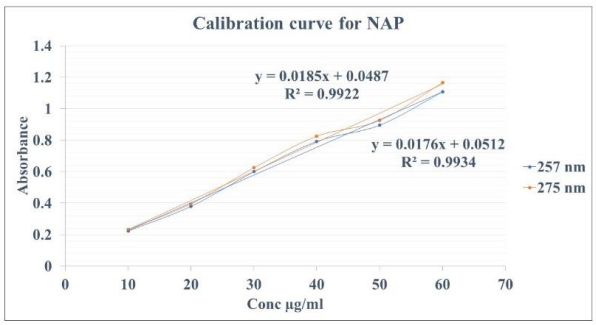


Figure 3a. Linearity of NAP and

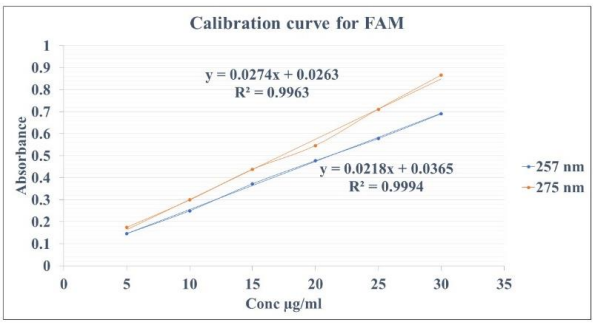


Figure 3b. Linearity of FAM

Table 1: Linearity values for NAP and FAM

Naproxen					**Famotidine**				
	At 257nm		**At 275nm**			**At 257nm**		**At 275nm**	
Concentration (µg/ml)	**Mean absorbance± SD (n=3)**	**%RSD**	**Mean absorbance± SD (n=3)**	**%RSD**	**Concentration (µg/ml)**	**Mean absorbance± SD (n=3)**	**%RSD**	**Mean absorbance± SD (n=3)**	**%RSD**
10	0.224± 0.0005	0.26	0.232± 0.0030	1.29	5	0.146±0.0026	1.81	0.172±0.0025	1.45
20	0.381± 0.0032	0.85	0.396± 0.0036	0.91	10	0.249± 0.0020	0.87	0.299± 0.0043	1.47
30	0.60± 0.0065	1.09	0.625± 0.0023	0.37	15	0.372± 0.0060	1.62	0.438± 0.0041	1.41
40	0.793± 0.0049	1.02	0.826± 0.0011	0.12	20	0.477± 0.0036	0.76	0.546± 0.0075	1.38
50	0.896± 0.0049	0.55	0.926± 0.0010	1.08	25	0.578± 0.0096	1.66	0.711± 0.0065	0.92
60	1.108± 0.0020	0.18	1.167± 0.0052	0.45	30	0.691± 0.0010	0.13	0.865± 0.0055	0.64

Precision-

Precision is defined by the ICH as the degree of agreement between values acquired in duplicates by measuring quantities under specific conditions. The mean, standard deviation, and variance, or coefficient of variation, are used to determine the degree of dispersion for a group of individual measurements.

Aliquots of 1ml from working standard FAM stock solution (100 µg/ml) were suitably diluted to obtain 10 μg/mL solutions. Similarly, 20 μg/mL NAP solution was prepared by suitably diluting NAP working standard stock solution (100 µg/ml). Six replicates of laboratory standard mixture containing 10 μg/mL of FAM and 20 μg/mL NAP were prepared and analyzed using the proposed method and percent relative standard deviation (%RSD) was calculated (Table 2.).

Table 2: Precision for NAP and FAM

Drug Name	Sample No.	Conc (µg/ml)	At 257 nm					At 275 nm				
			Amount found (µg/ml)	Percentage obtained (%)	Average (%)	SD	%RSD	Amount found (µg/ml)	Percentage obtained (%)	Average (%)	SD	%RSD
FAM	1.	10	10.32	103.29	101.68	1.98	1.94	10.82	108.27	105.02	1.96	1.86
	2.	10	10.10	101.02				10.28	102.86			
	3.	10	10.44	104.43				10.44	104.48			
	4.	10	9.87	98.75				10.34	103.40			
	5.	10	10.10	101.02				10.50	105.02			
	6.	10	10.15	101.59				10.61	106.10			
NAP	1.	20	19.19	95.98	97.78	1.16	1.19	19.18	95.93	96.1	1.38	1.435935
	2.	20	19.38	96.90				19.44	97.20			
	3.	20	19.56	97.82				19.07	95.38			
	4.	20	19.65	98.27				19.04	95.20			
	5.	20	19.70	98.50				18.93	94.65			
	6.	20	19.83	99.19				19.66	98.30			

Accuracy-

Three different spiked solutions were prepared for determination of accuracy by the addition of known concentrations of standard drug stock solutions to the pre-analyzed sample. 50, 100 and 120% were the three levels selected for analyzing the accuracy of the proposed method. The procedure was repeated three times and the percent recovery was determined. Aliquots of 0.8, 1 and 1.2 ml from working standard NAP solution (100µg/ml) were added at 80, 100 and 120% levels to pre-analyzed 1 ml sample solution of NAP and aliquots of 0.4, 0.5 and 0.6 ml of working standard FAM solution (100µg/ml) were added at 80, 100 and 120% level to pre-analyzed 0.5 ml sample of FAM and were suitably diluted. Absorbances of diluted solutions were measured at 257nm and 275nm. The amounts of NAP and FAM were determined at each level and % recoveries were calculated by measuring the absorbance and substituting the values in equation (1) (Table3).

Table 3: Percent recovery for the spiked samples.

Drug	Spike level in %	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery± SD (n=3
Naproxen Sodium	80%	10	8	8.1	101.25±0.004
	100%	10	10	10.29	102.9±0.0034
	120%	10	12	11.81	98.50±0.0030
Famotidine	80%	5	4	4.79	99.66±0.0068
	100%	5	5	4.97	99.71±0.0020
	120%	5	6	6.14	101.90±0.002

Limit of Detection (LOD):

Limit of detection refers to the smallest amount of analyte in a sample that can be detected but not precisely measured by an analytical technique. Three sets of linearity curves were used to calculate the LOD.

$$LOD = 3.3*SD/Slope$$

Where SD= standard deviation of Y-intercept of three calibration curves.

Slope= the mean slope of the three calibration curves.

Limit of quantification (LOQ):

The limit of quantification refers to the smallest amount of analyte that can be quantified but not detected by an analytical method. The LOQ was calculated using a series of three calibration curves.

LOQ was calculated by,

LOQ= 10*SD/Slope

Where SD= standard deviation of Y-intercept of three calibration curves.

Slope = the mean slope of the three calibration curves.

RESULTS AND DISCUSSIONS-

Naproxen being a BCS class II drug is insoluble in water. So saturation solubility study was carried out for the select of a suitable solvent. Based on the saturation solubility data, 2% SLS (pH 1.4) was chosen as a solvent for the analysis. Overlying spectra of both the drugs revealed absorbance maxima (λ_{max}) of 228, 275 nm and is the absorptive wavelength of 257 nm. Linearity refers to an analytical procedure's ability to yield results proportional to the concentration of the analyte within a given range. The suggested analytical method was found to be linear at 257 nm and 275 nm for NAP and FAM in the range of 10-60 µg/ml and 5-30 µg/ml, respectively (figure 3a and figure 3b). The % recovery for the actual and spiked amounts of the solutions was calculated to determine the accuracy of the established analytical procedure. The recovery data obtained is depicted in table 3. The closeness of agreement between values obtained by repeated measurements of a quantity under specific conditions is the precision of an analytical method. The precision of the method was confirmed by intermediate precision. % RSD value was found to be less than 2%. So the method was found to be precise. The results were shown in table 2. The values for LOD and LOQ were found to be lower which suggests that the developed method was quite sensitive. A summary of Validation parameters is depicted in Table 4.

Table 4: Summary of regression characteristics and validation parameters

Parameters	NAP		FAM	
	257nm	**275nm**	**257nm**	**275nm**
Beer's law (µg/ml)	10-60	10-60	5-30	5-30
Regression equation ($y = mx + c$)	0.0176	0.0185	0.0218	0.0274
Slope (m)				
Intercept (c)	0.0512	0.0487	0.0365	0.0263
Correlation coefficient (r^2)	0.9934	0.9922	0.9994	0.9963
Standard deviation (SD)	0.0030	0.0025	0.0018	0.0043
LOD (µg/ml)	0.562	0.089	0.276	0.650
LOQ (µg/ml)	1.721	0.270	0.837	1.97

CONCLUSION-

A UV spectroscopic Q-absorption ratio approach was devised and tested for the simultaneous analysis of NAP and FAM in combination. The method can be used to estimate NAP and FAM in laboratory samples. Based on the proposed optimum conditions, validation studies were carried out to determine the method's performance. Different validation parameters were tested, including linearity, accuracy, LOD, LOQ, precision, and repeatability. Linearity studies were carried out to determine slope, correlation coefficient, intercept, range and SD. The correlation coefficient was calculated from the regression line. The results for the precision parameter were expressed as the percentage relative standard deviation. %RSD values of both the drugs during the interday and intraday precision study were less than 2% therefore, meeting the acceptance limit. During accuracy studies, each solution was tested three times and average recovery at each level was determined. The percent recovery was found in the range of 90-110%. All the observations of tested validation parameters were found to be within acceptable limits.

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