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Development And Validation Of Multiwavelength Method For Simultaneous Estimation Of Nadifloxacin And Ibuprofen In Formulated Hydrogel

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1. Abstract: This paper describes validated Multiwavelength Spectrophotometric method for the simultaneous estimation Nadifloxacin and Ibuprofen in hydrogel formulation. The solutions of standard and sample were prepared in methanol water. Quantitative determination of the drugs was performed at 222 nm, 280nm and at 294 nm (N = 1; Δλ = 1) for Ibuprofen and Nadifloxacin, respectively. Proposed method was evaluated for the different validation parameters. The specificity test showed that there was no interference from excipients commonly found in the commercial pharmaceutical formulations at the analytical wavelengths of Ibuprofen and Nadifloxacin. Quantification was achieved over the concentration range of 2-20 µg/ ml for Nadifloxacin and for Ibuprofen respectively. The correlation coefficient of Nadifloxacin and Ibuprofen was found to be 0.9973 and 0.9983 respectively. The mean recovery was 100.25 and 99.37 % for Nadifloxacin and Ibuprofen, respectively. This method is simple, precise, and sensitive and applicable for the simultaneous determination of Nadifloxacin and Ibuprofen in hydrogel formulation.
Keywords: Nadifloxacin, Ibuprofen, Multiwavelength method, UV Spectrophotometry.

2. INTRODUCTION AND EXPERIMENTAL

Combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. Market survey revealed that, day by day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug. The analytical chemistry hence has challenge in developing the methods for their analysis with the help of number of analytical techniques which are available for the estimation of the drugs and their combination. Analytical monitoring of pharmaceutical product or specific ingredients within the product is necessary to ensure its safety and efficacy throughout the shelf life, including storage, distribution and use. I market such a combination of formulation not available yet so our aim is to prepare the multicomponent hydrogel formulation containing Nadifloxacin and Ibuprofen and analyzed it.

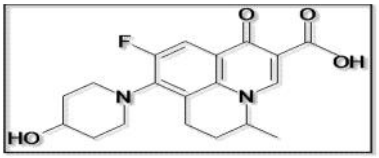

Figure 1. Structure of nadifloxacin

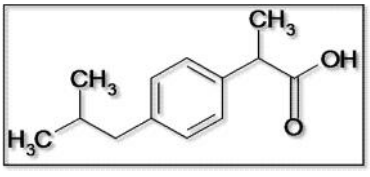

Figure 2. Structure of Ibuprofen.

Nadifloxacin (NAD), designated chemically as 9-fluoro-6,7-dihydro-8(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo-(ij)-quinolizine-2-carboxylic acid (Figure 1), is a second generation broad spectrum fluoroquinolone with improved activity against Gram-positive and Gram-negative bacteria, including *S. aureus, P. acnes and S. epidermidis*. The bactericidal action of nadifloxacin is mediated by inhibiting the formation of supercoiled DNA by DNA gyrase (topoisomerase II), an enzyme responsible for bacterial DNA replication[(1)]. It is used for the treatment of bacterial skin infection and acne vulgaris [(2-6)].

The 2-arylproprionic acid derivative, Ibuprofen [RS-2-(4-isobutyl-phenyl)propionic acid], is one of the most potent orally active antipyretic, analgesic and nonsteroidal anti-inflammatory drug (NSAID) used extensively in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions. This compound is characterized by a better tolerability compared with other NSAIDs[(7)]. Ibuprofen contains a chiral carbon atom on the propionic acid side-chain, therefore it exists as two enantiomers. It is usually marketed as a 50:50 mixture of the *S*- and *R* enantiomers, even if it is known that the pharmacological activity is due almost exclusively to the *S*- enantiomer[(8-13)].

2.1. MATERIALS

Working standards of pharmaceutical grade Nadifloxacin and Ibuprofen (IP) were procured from (Elder Pharmaceutical, Navi-Mumbai). Fixed dose combination hydrogel containing 1% NAD and 5% IBU was prepared in-house. All chemicals and reagents were of analytical grade and purchased from Loba Chemicals, Mumbai, Maharashtra.

2.2. INSTRUMENTATION

The present work was carried out on JASCO UV/Vis spectrophotometer, model no. V-630 with 1 cm matched quartz cells was used for experiments. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200- 400 nm.

2.3. SELECTION OF SOLVENT SYSTEM

NAD and IBU were dissolved separately in Methanol water solvent. The absorbances of NAD and IBU at respective wavelengths were determined.

2.4.PREPARATION OF NAD AND IBU STANDARD STOCK SOLUTIONS

NAD and IBU 10 mg each were accurately weighed and dissolved separately in Methanol: Water; 20:80 in 100 ml of volumetric flask. Shake and sonicate it for 10 min, adjust the final volume with same solvent to 100 ml with to get a concentration of 100µg/ml. These were used as stock solutions. In order to made 10µg/ml of each solution, 1 ml of stock solution diluted with water system in 10 ml of volumetric flask.

2.5. SELECTION OF WAVELENGTH (λ_{max})

The above prepared stock solutions of NAD and IBU were scanned in the range of 200-400nm to determine the wavelength of maximum absorption for both the drugs. NAD showed absorption maxima at 294 nm whereas IBU showed at 222 nm.

For estimation of IBU, multi-wavelength spectrophotometric method employing 220 nm and 280 nm as analytical wavelengths were used; the two wavelengths were chosen to eliminate interference of NAD at the sampling wavelength of IBU by taking the difference in the absorbance at the two wavelengths. For estimation of NAD, 294 nm was selected as the analytical wavelength, as IBU showed no absorption at this wavelength. In the multiwavelength method developed for simultaneous estimation of IBU and NAD, the wavelengths were selected from the overlain spectra shown in Figure [3].

2.5. PREPARATION OF CALIBRATION CURVE

From the respective stock solution (100µg/ml) different concentration of 2, 4, 6, 8, 10, 12,14,16,18, and 20µg/ml NAD and IBU were prepared and scanned in UV region. Their absorbances were noted at their above selected respective wavelength, calibration curve were plotted as absorbance v/s concentration and their linearity range was determined shown by Figure [4] for NAD & Figure [5] for IBU.

2.6. ANALYSIS OF FORMULATION:

A quantity of cream equivalent to 10 mg of NAD and 50 mg IBU (1 g of cream) was weighed and transferred to a 100 ml volumetric flask and added 30 ml of methanol: water; 20:80, the solution was warmed for 5-10 mins, ultrasonicated for 20 mins and volume was made up to the mark with same solvent. The solution was filtered using Whatman paper No. 41. From this solution, appropriate volume of 0.5 ml was transferred in 10 ml volumetric flask and volume was adjusted up to the mark with same solvent to get the concentration of 5 µg/ml of NAD and 25µg/ml of IBU. Suitable aliquots were prepared; scanned in UV region and absorbance was noted at selected wavelengths.

2.7. METHOD VALIDATION

The developed method was validated in terms of parameters like accuracy, precision, linearity, limit of detection and limit of quantitation shown in Table 2 – 8. [ICH (Q2B) guideline]

2.7.1. LINEARITY

The linearity for spectrophotometric method was established in the concentration of 2-20µg/ml for NAD and 2-20µg/ml for IBU. Data of regression summarized in **Table 2.**

2.7.2. ACCURACY

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation, a known quantity of standard NAD and IBU was added at 80%, 100% and 120% level and the contents were re-analyzed by the proposed method. The % recovery and %RSD (Relative Standard Deviation) were calculated shown in Table 4.

2.7.3. PRECISION

Precision studies were performed by preparing the standards three times and measuring the absorbance of drugs at selected wavelengths. Interday & intraday precision carried out according to ICH (Q2B) guideline.

2.7.4. LIMIT OF DETECTION

LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The result of limit of detection for NAD and IBU has been shown in Table 8.

2.7.5 LIMIT OF QUANTITATION

LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The result of LOQ for NAD and IBU has been shown in Table 8.

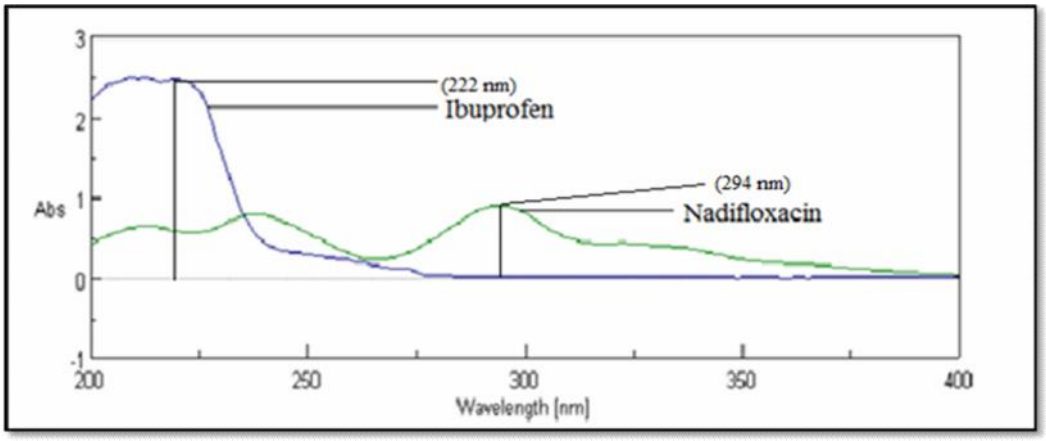

Figure 3. Overlain spectra of Nadifloxacin & Ibuprofen

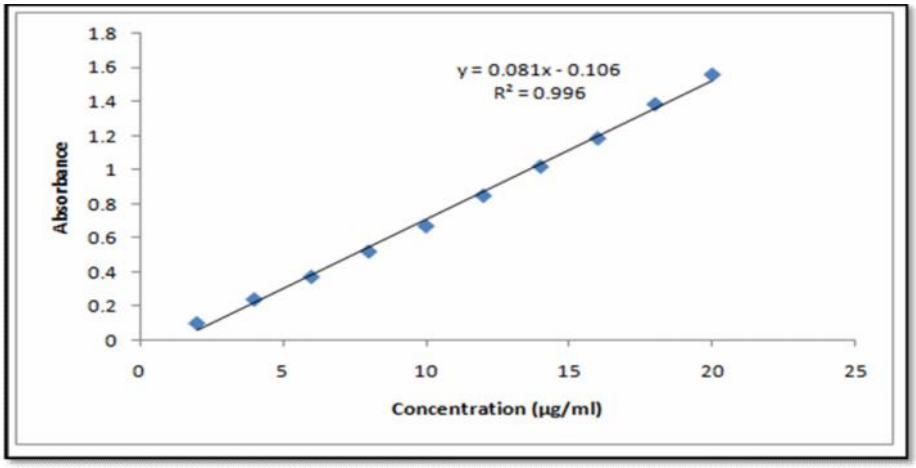

Figure 4. Calibration curve of Nadifloxacin at 294nm (2-20µg/ml)

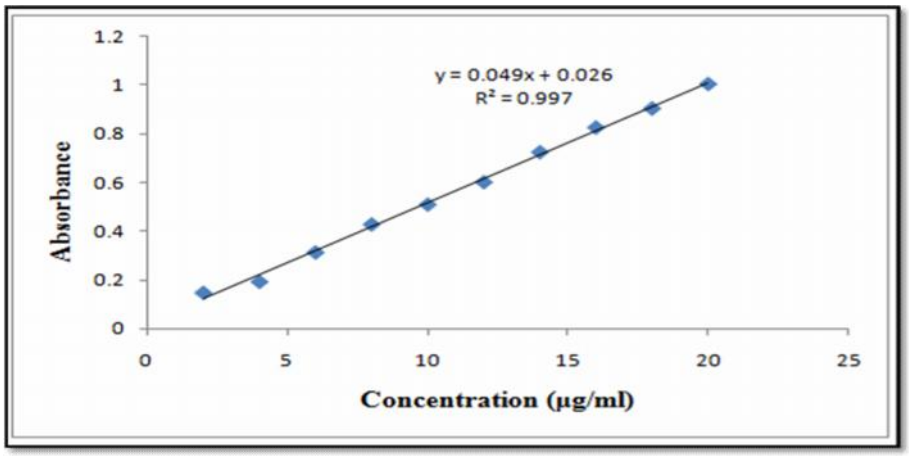

Figure 5. Calibration curve of Ibuprofen at 222 nm (2-20ug/ml)

Table 1 Optimum parameters

Parameter	Nadifloxacin	Ibuprofen
Λmax	294	222
Beers law Range	2-20 µg/ml	2-20 µg/ml
Equation	Y= A + B*C	Y= A + B*C
Slope	0.0817	0.049133
Intercept	-0.10809	0.026493
Correlation coefficient	0.9984	0.998648

*Y= A + B*C, where C is the concentration in µg/ml and Y is absorbance unit.

Table 2 Calibration curve of NAD & IBU

Conc. Of NAD (µg/ml)	Absorbance at 294 nm*	%RSD	Conc. Of IBU (µg/ml)	Absorbance at 222 nm*	%RSD
2	0.0966±0.000458	0.4743	2	0.1496±0.0003	0.2041
4	0.2394±0.0007	0.2923	4	0.1954±0.0004	0.2130
6	0.3726±0.001552	0.4166	6	0.3157±0.0008	0.2579
8	0.5199±0.001563	0.3006	8	0.4307±0.00045	0.1063
10	0.6705±0.000569	0.0848	10	0.5126±0.0010	0.2048
12	0.8476±0.001997	0.2356	12	0.6035±0.0014	0.2414
14	1.0192±0.001686	0.1654	14	0.7258±0.00055	0.0758
16	1.1867±0.003456	0.2912	16	0.8253±0.00186	0.2254
18	1.3843±0.004451	0.3215	18	0.9051±0.001	0.1106
20	1.5596±0.001442	0.0924	20	1.004±0.00147	0.1467

Absorbance at 294/222 nm* indicates mean of 3 observations.

Table 3 Analysis of Hydrogel formation

Drug	Amount (µg/ml)		% Label claim estimated*	% RSD
	Labeled	Found		
NAD	5.0	5.02	100.25 ±0.281	0.281
IBU	25.0	25.95	99.37±1.2	1.23

% Label claim estimated* indicates mean of 3 observations

Table 4 Recovery study

Level	% Recovery*		% RSD	
	NAD	IBU	NAD	IBU
80%	100.46±0.5543	99.76±1.024	0.555	1.027
100%	101.51 ±0.4325	99.51±0.927	0.426	0.831
120%	100.26± 0.9652	99.81±1.721	0.965	1.730

% Recovery* indicates mean of 3 observations.

Table 5 Results of Interday precision

Time	% Label claim estimated*		% RSD	
	NAD	IBU	NAD	IBU
T-1	100.43±0.625	99.53±0.867	0.626	0.868
T-2	99.95±1.231	99.68±1.524	1.232	1.524
T-3	101.51±0.615	99.91±0.098	0.616	0.098

% Label claim estimated* indicat.es mean of 3 observations.

Table 6 Results of Intraday precision

Time	% Label claim estimated*		% RSD	
	NAD	IBU	NAD	IBU
D-1	100.73±0.522	99.83±0.652	0.761	0.625
D-2	101.89±0.083	99.79±0.860	0.861	0.871
D-3	99.51±1.051	99.92±1.007	1.009	1.007

% Label claim estimated* indicates mean of 3 observations.

Table 7 Results of Precision

Conc.µg/ml	Absorbance		% RSD	
	NAD	IBU	NAD	IBU
8	0.0580	0.0599	0.0451	0.0765
	0.0585	0.0602		
	0.0579	0.0608		
10	0.0698	0.0736	0.0931	0.0541
	0.0701	0.0730		
	0.0691	0.0733		

Table 8 Results of LOD & LOQ

LOD (µg/ml)		LOQ (µg/ml)	
NAD	IBU	NAD	IBU
0.151	0.096	0.439	0.261

3. RESULTS AND DISCUSSION

3.1. OPTIMIZATION OF SOLVENT

As NAD and IBU is soluble in methanol water, various solvent mixtures containing methanol and water in different ratio were tried and different spectra pattern were studied. Finally Methanol: Water (20:80) is finalized as solvent system.

3.2. LINEARITY

In proposed Multiwavelength methods, at the selected range of λmax, both the drug solutions follow the Beer's Law in the concentration range of 2 - 20µg/ml (for NAD) and 2 - 20 ug/ml (for IBU). The values of coefficient of correlation were found to nearly equal to 0.999 for both NAD and IBU, shown in Fig 3 and 4.

3.3. ACCURACY

The accuracy of the developed methods was tested by standard addition method at the level of 80%, 100% and 120%. The percentage of recovery, lower values of standard deviation and relative standard deviation (< 2) indicates the accuracy of the proposed method was accurate methods Table 4.

3.4. PRECISION

The repeatability, intra-day and inter-day assay of the formulations by the proposed methods were

found to be suitable with very low values of relative standard deviation. This justifies the reproducibility and repeatability of the proposed methods, shown in Table 5, 6 and 7.

3.5. LOD AND LOQ

LOD for NAD and IBU were found to be 0.151µg/ml and 0.096µg/ml respectively. LOQ for NAD and IBU were found to be 0.261µg/ml and 0.429µg/ml respectively. These data show that microgram quantity of both drugs can be accurately determined shown in Table 8.

4. CONCLUSION

Simple multiwavelength UV spectrophotometric methods were developed for the simultaneous determination of NAD and IBU in hydrogel dosage form. To the best of our knowledge, the present study is the first report for the purpose. The present method succeeded in adopting a simple sample preparation and achieved satisfactory percentage recovery and therefore it can be concluded that use of this method can save analysis time and money. The proposed method is accurate and precise for the determination of NAD and IBU in combined form. Hence, it can be employed for routine analysis in Quality Control Laboratories.

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