Stamford Journal of Pharmaceutical Sciences

Development of an Assay Method for Simultaneous Determination of Ciprofloxacin and Naproxen by UV Spectrophotometric Method

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Original Research Article

ABSTRACT

The Objective of the present study was to develop a simple, reproducible and economical spectrophotometric method for simultaneous determination of Ciprofloxacin and Naproxen. Both Drugs obey Beer's law in the range of 1-5µg/ml concentration. The Standard curve of Ciprofloxacin and Naproxen in the media of 0.1N HCl, Distilled water and Phosphate buffer are obtained by plotting absorbance versus concentration where calibration curve was found to be linear (R^2>0.99) with optimum value of standard error for the entire analytical medium used. The plot of the residuals was normally distributed around the regression line, which reflects the accuracy of the method. For simultaneous determination of Ciprofloxacin and Naproxen, the linear plot was found in the media of distilled water and 0.1N HCl acid. The maximum absorbance was found in the media of distilled water for Ciprofloxacin at wavelength 278nm and for Naproxen at 228nm. The calibration curve was to be linear for Ciprofloxacin as R^2 >0.99 and for Naproxen as R^2 >0.99.The maximum absorbance was found in the media of 0.1N HCl for Ciprofloxacin at wavelength 277nm and for Naproxen at 228nm. The calibration curve was to be linear for Ciprofloxacin as R^2 >0.98 and for Naproxen as R^2 >0.99.

***Key words*:** Ciprofloxacin, Naproxen, Spectrophotometric analysis, Simultaneous equation method.

INTRODUCTION

Naproxen (NAP) (Figure 1-I) is chemically (+)-(S) - 6-methoxy- α-methyl-2-naphthalenacetic acid (USP 2004). It is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness (Haque *et al.*, 2008) and works by inhibiting both the COX-1 and COX-2 enzymes that cause inflammation and pain in the body (Sutradhar et al, 2011). Ciprofloxacin (Figure 1-II) is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (Patric, 2001). Fluoroquinolones such as ciprofloxacin are known as effective antibiotics with excellent activities against numerous respiratory tract pathogens. They are applied for the topical treatment of recurrent purulent otitis media, a common and often bacterial driven inflammatory disease of the middle ear. Moreover, similar to macrolides, fluoroquinolones have been reported to exert immunomodulatory effects on various cell types (Sachse *et al.*, 2008; Dalhoff, 2005). It inhibits bacterial DNA gyrase, an enzyme responsible for counteracting excessive supercoiling of DNA during replication or transcription (Hardman and Limbard, 2001). Non-steroidal anti-inflammatory drugs (NSAIDs) remain the most commonly used drugs for treating the symptoms of Osteoarthritis and several other inflammatory diseases, despite their significant gastrointestinal (GI) and cardiovascular toxicity (Grosser *et al.*, 2006; Wallace, 2008). Ciprofloxacin is one of the few broad spectrum antibacterials available in both intravenous and

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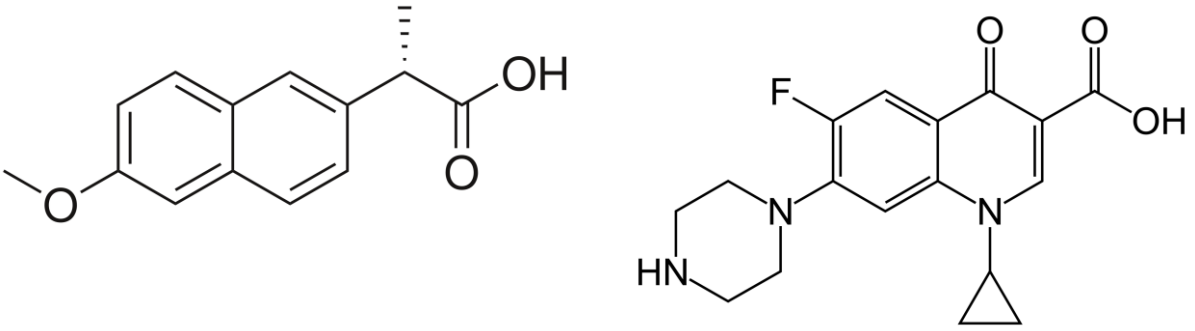

(I) **(II)**

Figure 1. Chemical structure of (I) Naproxen and (II) Ciprofloxacin.

oral formulations. In this respect, it offers the potential for cost savings with sequential intravenous and oral therapy in appropriately selected patients and may allow early discharge from hospital in some instances. In conclusion, ciprofloxacin has retained its excellent activity against most Gram-negative bacteria, and fulfilled its potential as an important antibacterial drug in the treatment of a wide range of infections. Rational prescribing will help to ensure the continued clinical usefulness of this valuable antimicrobial drug. The combination may be useful in patients with concurrent infection and rheumatic pain and in such cases the simultaneous determination of ciprofloxacin and naproxen using a simple analytical method is required. If a suitable method for specific need is not available then it becomes essential to develop simple sensitive, accurate, precise and reproducible method for the estimation of drug samples.

UV Spectroscopy method is one of the instrumental analytical methods that are widely used in pharmaceutical industries for the assay of pharmaceutical products, because it is simple, easy, less time consuming and an economical method (Fatema *et al.*, 2010). Literature survey has revealed that few HPLC and TLC methods have been reported for determination of Ciprofloxacin and Naproxen (Krzek et al, 2005; Imre et al, 2005; Kassab et al, 2005; Amini et al, 2004) as single component in bulk and in formulations. Also, not a single UV and HPTLC method is reported for simultaneous analysis of Ciprofloxacin and Naproxen. A successful attempt has been made to estimate two drugs simultaneously by spectrophotometric analysis. The objective of the investigation was to develop a methodology for the estimation of the combined dosage form by simultaneous UV spectrophotometric method.

MATERIALS & METHODS

Chemicals and Reagents: Reference standard of Ciprofloxacin & Naproxen were collected from Incepta Pharmaceuticals Ltd, Bangladesh. Dipotassium hydrogen phosphate and potassium orthophosphate were purchased from BDH (UK) Ltd. All other ingredients used were of analytical grade. **Instruments:** UV-Visible spectrophotometer (HACH, model- DR/4000 UV-VIS), Electric balance (Denver instrument, model- M-310), Digital pH meter (LIDA instrument, model- PHS-25)

Preparation of phosphate buffer of pH 2.5

700 mM buffer solution was prepared by adding 16.3308 g KH_2PO_4 and 3.4836 g K_2HPO_4 in 200 ml distilled water. pH of the solution was adjusted to 2.5 by H_3PO_4.

Preparation of phosphate buffer of pH 7.4

700 mM buffer solution was prepared by adding 2.7218 g KH_2PO_4 and 8.709 g K_2HPO_4 in 200 ml distilled water. pH of the solution was adjusted to 7.4 by NaOH.

Preparation of 0.1N HCl acid:

0.83 ml of 37% pure concentrated HCl was taken in a 100 ml volumetric flask. The volume was

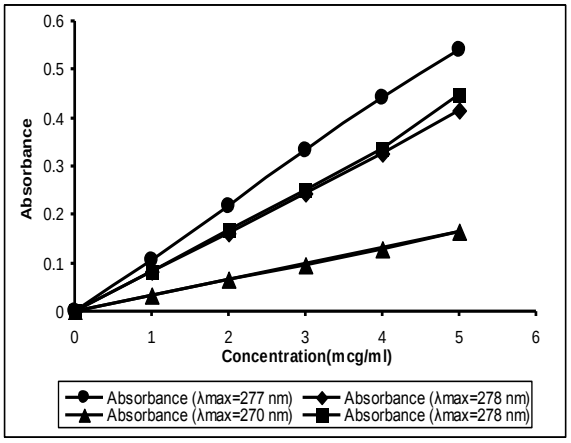

Figure 2. Calibration curve of Ciprofloxacin in the media of 0.1 N HCl, Distilled water, Phosphate Buffer pH 7.4 and pH 2.5.

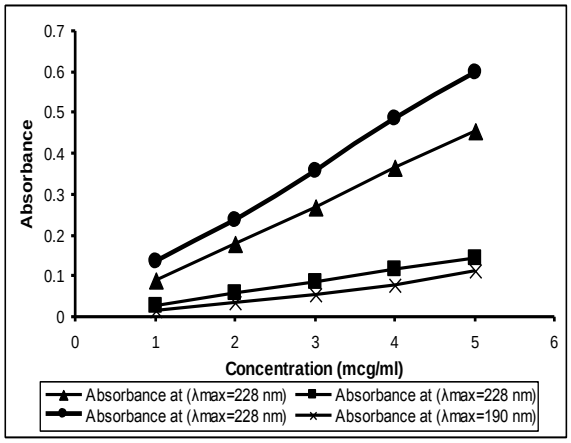

Figure 3. Calibration curve of Naproxen in the media of Distilled water, 0.1N HCl, Phosphate buffer pH 7.4 and pH 2.5.

made up with distilled water and the content is thoroughly mixed it by shaking and approximately 0.1N HCl solution was prepared.

Preparation of solution of Ciprofloxacin at different concentrations and scanning wavelength at different media

50µg/ml of stock solution was prepared by dissolving 5mg of Ciprofloxacin in 100ml of phosphate buffer having pH of 2.5 and pH 7.4 respectively , in 0.1N HCl and in Distilled water. Standard solution of various concentrations (1, 2, 3, 4, 5µg/ml) were prepared by dilution from each stock solution. The wavelength of maximum emission (λmax) of Ciprofloxacin in each media was found by scanning them over the UV range of 190nm to 400nm. Concentration vs. absorbance was plotted and standard curve was prepared by scanning the standard solutions of Ciprofloxacin at 278nm in the media of Buffer pH 2.5, at 270nm in the media of Buffer pH 7.4, at 277nm in the media of 0.1N HCl, at 278nm in the media of Distilled water, presented in Figure 2.

Preparation of solution of Naproxen at different concentrations and scanning wavelength at different media

50µg/ml of stock solution was prepared by dissolving 5mg of Naproxen in 100ml of phosphate buffer having pH of 2.5 and pH 7.4 respectively, in 0.1N HCl and in Distilled water. Standard solution of various concentrations (1, 2, 3, 4, 5µg/ml) were prepared by dilution from each stock solution. The wavelength of maximum emission (λmax) of Ciprofloxacin in each media were found by scanning them over the UV range of 190nm to 400nm. Concentration vs. absorbance was plotted and standard curve was prepared by scanning the wavelength (λ_{max}) of Naproxen at 228nm in the media of Buffer pH 7.4, in the media of 0.1N HCl and in the media of Distilled water presented in Figure 3.

Preparation of Standard curve for Simultaneous determination of Ciprofloxacin and Naproxen in the media of 0.1N HCl acid

In combination, Absorbance of different concentrations were taken at the wavelength (λmax) of 277nm of this particular medium for Ciprofloxacin and for Naproxen, absorbance of different concentrations were taken at the wavelength (λmax) of 228nm.Concentrations vs. absorbance were plotted for both drugs and standard curve was prepared and presented in Figure 4.

Preparation of Standard curve for Simultaneous determination of Ciprofloxacin and Naproxen in the media of distilled water

In combination, Absorbance of different concentrations were taken at the wavelength (λmax) of 278nm of this particular medium for Ciprofloxacin and for Naproxen, absorbance of different concentrations were taken at the wavelength (λmax) of 228nm. Concentrations

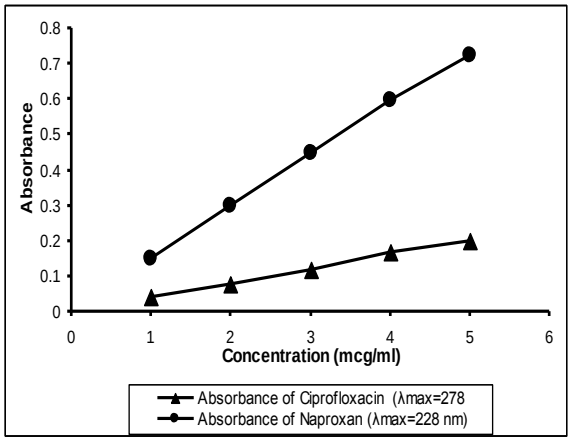

Figure 4. Calibration curve of Ciprofloxacin and Naproxen in the media of distilled water.

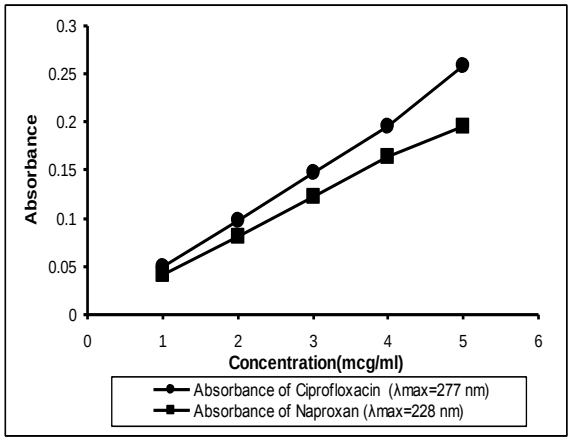

Figure 5. Calibration curve of Ciprofloxacin and Naproxen in the media of 0.1 N HCl.

vs. absorbance were plotted for both drugs and standard curve was prepared and presented in Figure 5.

RESULTS AND DISCUSSION

For Ciprofloxacin, the maximum absorbance was found at wavelength 277nm for 0.1N HCl acid, at wavelength 278nm for Distilled water, at wavelength 270nm for pH 7.4 and at wavelength 278nm for pH 2.5. Naproxen represents the maximum absorbance at wavelength 228nm for 0.1N HCl acid, at wavelength 228nm for Distilled water, at wavelength 228nm for pH 7.4 and at wavelength 190nm for pH 2.5. The standard curve of Ciprofloxacin and Naproxen in the media of 0.1N HCl, Distilled water and phosphate buffer were obtained by plotting absorbance versus concentration where the calibration curve was found to be linear (R^2 >0.99) with optimum value for Ciprofloxacin of standard error for the entire analytical medium used. The plot of the residuals was normally distributed around the regression line, which reflects the accuracy of the method. The extinction co-efficient values were calculated with Lambert-Beer's law for 0.1N HCl, Distilled water, & phosphate buffer media. The values of regression equation, correlation coefficient (R^2) value and standard error of the regression line of Ciprofloxacin and Naproxen are presented respectively in Table 1 to Table 3 and Table 4 to Table 6. For simultaneous determination of Ciprofloxacin and Naproxen, the linear plot was found in the media of distilled water and 0.1N HCl acid. At different concentrations the maximum absorbance was found in the media of distilled water for Ciprofloxacin at wavelength 278nm and for Naproxen at 228nm represented in Table 7 and in the media of 0.1N HCl for Ciprofloxacin at wavelength 277nm and for Naproxen at 228nm represented in Table 8. Statistical parameters of simultaneous determination of Ciprofloxacin and Naproxen in the media of Distilled water and 0.1N HCl were represented in Table 9, Table 10 and in Table 11 respectively.

From the proposed method, it was found that Ciprofloxacin and Naproxen obeys linearity within the concentration range 1-5µg/ml both individual and in combination. Under experimental conditions described, calibration curve was obtained by plotting absorbance versus concentration with good correlation co-efficient (R^2>0.99) indicates the linearity of the method with optimum value of standard error for the entire analytical medium used.

CONCLUSION

Present study indicates that, it is possible to determine the content of ciprofloxacin and naproxen by UV spectrophotometric method. The functional equation and the extinction coefficient values derived from the calibration

Table 1. Absorbance of Ciprofloxacin at different concentrations in the media of 0.1 N HCl, Distilled water, Buffer7.4, Buffer 2.5.

Concentration (µg/mL)	Absorbance (0.1 N HCl at λmax=277nm)	Absorbance (Distilled Water at λmax=278nm)	Absorbance (Buffer-7.4 at λmax=270nm)	Absorbance (Buffer-2.5 at λmax=278nm)
1	0.107	0.081	0.033	0.083
2	0.219	0.163	0.065	0.167
3	0.332	0.243	0.095	0.251
4	0.441	0.328	0.13	0.337
5	0.542	0.414	0.165	0.450

Table 2. Regression Parameters of Ciprofloxacin.

Media	0.1 N HCl	Distilled water	Buffer pH 7.4	Buffer pH 2.5
λmax	277nm	278nm	270nm	278nm
Extinction coefficient	42410.85±0.20	31506.70±0.154	12556.12±0.061	32333.66±0.134
RSD% for Extinction coefficient ε	0.6230	0.6287	0.6398	0.5329

Table 3. λmax and extinction co-efficient values (M^{-1} cm^{-1}) of ciprofloxacin.

Media	Regression Equation	Correlation co-efficient (r)	Standard error (s)
Distilled water	Y= 0.0831x-0.0035	0.9998	0.0691
0.1 N HCl acid	Y = 0.1092x + 0.0006	0 .9995	0.0914
Buffer 7.4	Y= 0.0329x-0.001	0.9990	0.0274
Buffer 2.5	Y= 0.0904x-0.0136	0.9959	0.0601

Table 4. Naproxen in the media of Distilled water, 0.1N HCl, Phosphate buffer pH 7.4 and pH 2.5.

Concentration (µg/mL)	Absorbance at (λmax=228nm)	Absorbance at (λmax=228nm)	Absorbance at (λmax=228nm)	Absorbance at (λmax=190nm)
1	0.091	0.029	0.138	0.015
2	0.178	0.058	0.239	0.035
3	0.269	0.087	0.359	0.054
4	0.364	0.116	0.485	0.078
5	0.455	0.145	0.599	0.111

Table 5. Regression Parameters of Naproxen.

Media	Regression Equation	Correlation co-efficient (r)	Standard error (s)
Distilled water	Y= 0.091x-0.0013	0.9999	0.0646
0.1 N HCl acid	Y = 0.029x-17	0.9999	0.020
Buffer 7.4	Y= 0.1187x +0.0065	0.9999	0.082
Buffer 2.5	Not satisfactory	Not satisfactory	Not satisfactory

Table 6. λmax and extinction co-efficient values (M^{-1} cm^{-1}) of Naproxen.

Media	0.1 N HCl	Distilled water	Buffer 7.4	Buffer 2.5
λmax	228nm	228nm	228nm	190nm
ε(M^{-1} cm^{-1})	6677.79±0.04585	20788.93±0.14453	27651.56±0.18479	Not satisfactory
RSD% for ε	0.5270	0.5325	0.5076	Not satisfactory

Table 7. Absorbance of Ciprofloxacin and Naproxen in the media of Distilled water.

Concentration(μg/mL)	Absorbance of Ciprofloxacin (λmax=278nm)	Absorbance of Naproxen (λmax=228nm)
1	0.039	0.149
2	0.079	0.298
3	0.119	0.447
4	0.165	0.596
5	0.201	0.722

Table 8. Absorbance of Ciprofloxacin and Naproxen in the media of 0.1 N HCl acid.

Concentration(μg/mL)	Absorbance of Ciprofloxacin(λmax=277nm)	Absorbance of Naproxen(λmax=228nm)
1	0.049	0.041
2	0.098	0.082
3	0.147	0.123
4	0.196	0.164
5	0.259	0.196

Table 9. Statistical Parameters for UV-spectrophotometric determination of Ciprofloxacin & Naproxen in Distilled water.

Regression Parameters	Ciprofloxacin	Naproxen
Regression Equation	Y= 0.1484x + 0.0092	Y= 0.041x – 0.0024
Correlation co-efficient (R)	0.999	0.999
Standard error (s)	0.0289	0.1047

Table 10. Statistical Parameters for UV-spectrophotometric determination of Ciprofloxacin & Naproxen in 0.1N HCl acid.

Regression Parameters	Ciprofloxacin	Naproxen
Regression Equation	Y= 0.049x + 0.02	Y= 0.041x + 17
Correlation co-efficient (R)	0.9999	0.9999
Standard error (s)	0.0346	0.0289

Table 11. λmax and extinction co-efficient values (M^{-1} cm^{-1}) of Ciprofloxacin & Naproxen.

Media	0.1 N HCl	Distilled water	0.1 N HCl	Distilled water
λmax	277nm	278nm	228nm	228nm
ε(M^{-1} cm^{-1})	18905.565 ± 0.077	15403.536 ± 0.076	9441.015 ± 0.064	34273.187± 0.234
RSD% for ε	0.527	0.034	0.289	0.104

curve of reference standard of the pure drugs will enable the analyst to determine the drug content in pharmaceutical dosage forms. The UV spectrophotometric method will certainly offer distinct advantage of simplicity, accuracy and sensitivity in analyzing pharmaceutical formulations of ciprofloxacin and naproxen. In the media of distilled water and 0.1N HCl acid the standard curve of both Ciprofloxacin and Naproxen showed linearity which concluded that it is possible to determine the content of both of these drugs by Ultraviolet spectroscopic method combinedly.

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