Zero order and First order derivative spectroscopic method for simultaneous estimation of Moxifloxacin hydrochloride and Ketorolac tromethamine in simulated tear fluid

Shashank Nayak N*1, U Srinivasa 2, Shwetha S Kamath K3, Shabaraya AR
1Dept of Pharmaceutics, Pacific university, Udaipur, Rajasthan, India
2Dept. of Pharmacognosy, Srinivas college of pharmacy, Mangalore, Karnataka, India
3Dept. of Pharmaceutics, Srinivas college of pharmacy, Mangalore, Karnataka, India
*shashknayak87@gmail.com

ABSTRACT

In this current work simultaneous estimation of Moxifloxacin HCL and Ketorolac tromethamine in simulated tear fluid was performed by using UV spectroscopy. The zero order derivative spectroscopy revealed that Moxifloxacin HCL had a λ max of 288 nm in Simulated tear fluid(STF) and Ketotorolac tromethamine had λ max of 322nm respectively. The linearity was found in the range of 2-10µg/ml for Moxifloxacin HCL and 2-14µg/ml for ketorolac tromethamine. The R² value was found to be 0.998 and slope y=0.089 for Moxifloxacin HCL and R² =0.998 and slope y=0.051 for Ketorolac tromethamine. The zero order spectrum was deravatized to first order derivative spectra for both the above mentioned drug. The first order derivative spectra of Moxifloxacin HCL and ketorolac tromethamine indicated that it had a zero crossing point (ZCP) at 286 nm and 328 nm. The absorbance of the mixture containing MOXI and KETO was found out at 286 nm and 328 nm which showed linearity and obeyed beers lamberts law.

Keywords: Moxifloxacin hydrochloride, Ketorolac tromethamine, Simulated tear fluid (STF),Zero crossing point(ZCP),Derivative spectroscopy, Zero order spectrum, First order spectrum.

INTRODUCTION

Moxifloxacin HCL is chemically 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octa-hydro-6H-pyrrolo[3,4-b]pyridine-6-y1]-4-oxo-3-quinoxalinecarboxylic acid mono hydrochloride. It comes under the fourth generation fluoroquinolone antibiotic. Moxifloxacin is a broad spectrum antibiotic which is active against both Gram-positive and Gram negative bacteria. The bactericidal action of moxifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. DNA gyrase is an essential enzyme that is involved in the replication, transcription and repair of bacterial DNA. Topoisomerase IV is an enzyme known to play a key role in the partitioning of the chromosomal DNA during bacterial cell division1-3.

Ketorolac tromethamine is chemically 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid. Ketorolac is a nonsteroidal anti-inflammatory drug (NSAID) chemically related to indomethacin and tolmetin. Ketorolac tromethamine is a racemic mixture [+]R-enantiomeric forms, with the S-form having analgesic activity. Its anti inflammatory effects are believed to be due to inhibition of both cylooxygenase-1 cylooxygenase-2 (COX-2) which leads to the inhibition of prostaglandin synthesis leading to decreased formation of precursors of prostaglandins and thromboxanes arachidonic acid4.

Figure 1:Structure of Moxifloxacin HCL

Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands. Although it

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was introduced more than thirty years ago, and has demonstrable advantages for the solution of specific analytical problems, this technique has been accepted only hesitantly, because of the initial lack of reasonably priced instrumentation and original limitation to the first derivative. However, in recent years, the introduction of electronic differentiation by a microcomputer interfaced with the spectrophotometer makes possible the plotting of the first, second or higher order derivatives of a spectrum with respect to wavelength. In this current study simultaneous estimation of Moxifloxacin HCL and Ketorolac tromethamine was done in simulated tear fluid by using using zero order and first order derivative spectroscopic methods.

**Materials and Methods**

**Instruments**
UV-Visible double beam spectrophotometer (SHIMADZU Co, Japan) with 1cm matched quartz cells, Micropipette of Variable volume 10-1000 μL (Gene Pete Co.) and Digital balance were used.

**Materials**
Moxifloxacin HCL was obtained as gift sample from micro labs Bangalore and Ketorolac tromethamine was obtained as gift sample from Ranbaxy goa. All other reagents were of analytical grade.

**Preparation of simulated tear fluid (STF)**
The simulated tear fluid was prepared by taking the following ingredients
- Sodium chloride-3.35 gms
- Sodium bicarbonate-1.0 gms
- Calcium chloride-0.04 gms
- Distilled water-500 ml

**Preparation of stock solution of Moxifloxacin HCL and Ketorolac tromethamine in STF**
100 mg of Moxifloxacin HCL and Ketorolac tromethamine was dissolved seperately in 50 ml of Simulated tear fluid(Stock A).From stock A take 1 ml diluted to 100 ml of simulated tear fluid (stock B).

**Preparation of working standard of Moxifloxacin HCL and ketorolac tromethamine**
From stock B of both Moxifloxacin HCL and Ketorolac tromethamine,(2-10µg/ml) working standard solution was prepared for Moxifloxacin HCL and (2-14µg/ml) working standard was prepared for Ketorolac tromethamine.

**Determination of λmax of Moxifloxacin HCL and construction of calibration curve in STF (zero order derivative spectra)**
Wavelength scan was done from 200-400 nm by using 2-10 µg/ml solution in double beam UV spectrophotometer Shimadzu (UV-1601) to get λmax and absorbance of the standard solution were noted down.

**Determination of λmax of Ketorolac tromethamine and construction of calibration curve in STF (Zero order derivative spectra)**
Wavelength scan was done from 200-400 nm by using 2-14 µg/ml solution in double beam UV spectrophotometer Shimadzu (UV-1601) to get λmax and absorbance of the standard solution were noted down.

**First order derivative spectra of Moxifloxacin HCL in STF**
The zero order spectrum of Moxifloxacin HCL was deravatized to first order spectrum in the software and ZCP of Moxifloxacin HCL and absorbance at ZCP of 2-10µg/ml was noted down.

**First order derivative spectra of Ketorolac tromethamine in STF**
The zero order spectrum of Ketorolac tromethamine was deravatized to first order spectrum in the software and ZCP of Ketorolac tromethamine and absorbance at ZCP of 2-14µg/ml was noted down.

**Determination of absorbance of Moxifloxacin HCL and Ketorolace tromethamine in mixture at ZCP**
The ZCP of Moxifloxacin HCL was used as absorbance maxima for Ketorolac tromethamine and ZCP of Ketorolac tromethamine was used as absorbance maxima for Moxifloxacin HCL and absorbance was noted down and linearity graphs were plotted.

**RESULTS AND DISCUSSION**

**λ max of Moxifloxacin HCL and calibration curve in STF (zero order derivative spectra)**
The λ max of Moxifloxacin HCL was found out at 288 nm(Figure 3)and linearity was in the range of 2-10 μg/ml and R² value was found to be 0.998 ,slope...
y=0.089. The absorbance was found at 288 nm is mentioned in figure no 4.

Figure 3-Overlay of λ max of Moxifloxacin HCL (2-10 µg/ml)

Figure 4-Standard graph of Moxifloxacin HCL in STF at 288nm

λ max of Ketorolac tromethamine and calibration curve in STF (zero order derivative spectra)
The λ max of Ketorolac tromethamine was found out at 322 nm (Figure 5) and linearity was in the range of 2-14 µg/ml and R² value was found to be 0.998, slope y=0.051. The absorbance was found at 322 nm is mentioned in figure no 6.

Figure 5-Overlay of λ max of ketorolac tromethamine (2-14 µg/ml)

Figure 6-Standard graph of Ketorolac tromethamine in STF at 322nm

First order derivative spectra of Moxifloxacin HCL in STF
The zero order spectra of Moxifloxacin HCL which had λmax of 288 nm was deravitized in software to first order spectra. The ZCP was found at 286 nm (Figure 7), so at this wavelength Moxifloxacin HCL showed almost zero absorbance (Figure 8) and R² was found to be 0.999 and slope y=-0.001x and this wavelength was selected for checking the absorbance of Ketorolac tromethamine in mixture.

Figure 7-ZCP of Moxifloxacin HCL at 286 nm

Figure 8-Absorbance of Moxifloxacin HCL at 286 nm (ZCP)
**First order derivative spectra of Ketorolac tromethamine in STF**

The zero order spectra of Ketorolac tromethamine which had λmax of 322 nm was derivatized in software to first order spectra. The ZCP was found at 328nm(Figure 9), so at this wavelength Ketorolac tromethamine showed almost zero absorbance(Figure 10) and R² was found to be 0.998 and slope y= -0.001x and this wavelength was selected for checking the absorbance of Moxifloxacin HCL in mixture.

![ZCP of Ketorolac tromethamine at 322 nm](image)

**Figure 9-ZCP of Ketorolac tromethamine at 322 nm**

![Absorbance of Ketorolac tromethamine at 328 nm](image)

**Figure 10-Absorbance of Ketorolac tromethamine at 328 nm(ZCP)**

**Determination of absorbance of Mixture of Moxifloxacin HCL and Ketorolac tromethamine at ZCP**

100 mg of Moxifloxacin HCL and Ketorolac tromethamine was dissolved in 50 ml of STF(Stock A). From this stock solution 1 ml was taken and diluted to 100 ml of STF(Stock B). From this stock B (2-14 µg/ml) solution was prepared and absorbance was noted down at 328 nm for Moxifloxacin HCL(Figure 11) and 286 nm for Ketorolac tromethamine (Figure 12)

![Absorbance of Moxifloxacin Hydrochloride at 328nm(ZCP of Keto)](image)

**Figure 11-Absorbance of Moxifloxacin HCL at 328 nm(ZCP of Keto)**

![Absorbance of Ketorolac Tromethamine at 286 nm(ZCP of MOXI)](image)

**Figure 12-Absorbance of Ketorolac tromethamine at 286 nm(ZCP of Moxi)**

**CONCLUSION**

In this current work first order derivative spectroscopy was used for analyzing the sample containing two drugs by considering the ZCP of individual drugs. So derivative spectroscopy can be used as best tool for analyzing Moxifloxacin HCL and Ketorolac tromethamine in mixture and formulation.

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