Derivative and Simultaneous Equation Methods for the Determination of Fluorometholone and Ketorolac in Ophthalmic Preparations

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Abstract

Introduction: Two new spectrophotometric techniques have been developed for the quantification of Fluorometholone and Ketorolac tromethamine in eye drops. **Materials and Methods:** First derivative method and simultaneous equation method were developed for the determination of Fluorometholone and Ketorolac tromethamine in both sodium acetate buffer (pH 4.0) and phosphate buffer (pH 4.0) reagent solutions. **Results and Discussion:** Fluorometholone and Ketorolac tromethamine has followed linearity over the concentration range 1-20 μg/mL sodium acetate buffer (pH 4.0) and 2-30 μg/mL in phosphate buffer. **Conclusion:** The methods were validated and applied for the simultaneous determination of Fluorometholone and Ketorolac tromethamine in the available ophthalmic preparations

Key words: First derivative method, fluorometholone, ketorolac tromethamine, simultaneous equation method, spectrophotometry, validation

INTRODUCTION

Ketorolac tromethamine (KT)[1] is freely soluble in methanol. An ophthalmic solution of Ketorolac is used to relieve itchiness and burning of seasonal allergies. Ketorolac acts as anti-inflammatory, antipyretic, and analgesic. KT is a non-selective COX inhibitor [Figure 1]. Fluorometholone[1] (FLM) is a synthetic glucocorticoid used for the treatment of inflammatory eye diseases. FLM is used in the treatment of steroid-responsive inflammatory conditions of the palpebral and bulbar conjunctiva, cornea, and anterior segment of the eye [Figure 2]. Very few spectrophotometric[2-3]and high-performance liquid chromatography (RP-HPLC)[4-7] methods have been developed for the combined formulations of FLM and KT. The authors have proposed two spectrophotometric methods - simultaneous equation method and first derivative simultaneous derivative method for the determination of FLM and KT in ophthalmic preparations in sodium acetate buffer (pH 4.0) and phosphate buffer (pH 4.0) and the methods are validated.[8]

MATERIALS AND METHODS

Chemicals and reagents

Stock solutions of both FLM and KT were prepared by dissolving 25 mg each in methanol in 25 ml volumetric flasks separately, and dilutions were made with sodium acetate buffer (pH 4.0) and phosphate buffer (pH 4.0). The combination of FLM and KT is available with brand name Eyetrust (Courtesy: Neiss labs Ltd., Mumbai) as eye drops containing FLM 0.1% and KT 0.5%.

Procedure

UV-1800 double beam UV-Vis spectrophotometer (Shimadzu) with a pair of 10 mm path length matched

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quartz cells is used for the study. All the solutions were scanned 200–400 nm with medium scanning speed. Two spectrophotometric techniques - simultaneous equation method (Method A and Method B) and first derivative method (Method C and Method D) were developed for the simultaneous determination of FLM and KT in both sodium acetate buffer and phosphate buffer (pH 4.0) reagents.

Method validation

Linearity

1–20 µg/mL FLM and 2–30 µg/mL KT solutions were prepared from their individual stock solutions separately in sodium acetate buffer(pH 4.0) (Method A) and phosphate buffer (pH 4.0) (Method B), respectively, and scanned against their reagent blank. The absorbance, as well as the absorptivity values, was calculated at the selected wavelengths for both Method A and Method B.

Simultaneous equation method

Adequate dilute solutions of FLM and KT were prepared from their stock solutions separately with sodium acetate buffer (pH 4.0) (Method A) and phosphate buffer (pH 4.0) (Method B) and scanned in quartz cuvettes in UV region (200–400 nm) as their solutions were colorless. The absorption spectra so obtained show λ_{max} at 242.13 nm for FLM and 322.56 nm for KT in sodium acetate buffer and at

Figure 1: Chemical structures of fluorometholone and ketorolac tromethamine

λ_{max} 242.19 nm for FLM and 322.23 nm for KT in phosphate buffer (pH 4.0), respectively. The absorptivity (ε) values were calculated from their individual spectral data and substituted in simultaneous equation where the individual concentrations of FLM and KT were calculated. Calibration curves were drawn by taking the concentration of the drug solution on the x-axis and the corresponding absorbance values on the y-axis at the selected wavelengths for both Method A and Method B. The individual concentrations of the drugs were determined by employing the absorptivity values in the simultaneous equations developed. The overlay absorption spectra of FLM and KT and their formulation in sodium acetate buffer and phosphate buffer pH 4.0 were shown in Figure 2.

Simultaneous first derivative method (D$_1$)

The individual zero-order absorption spectra of FLM and KT in sodium acetate buffer (Method C) were converted into the first-order derivative spectra with the help of inbuilt software. The overlay first-order derivative spectrum of FLM and KT in sodium acetate buffer was shown in Figure 3a. The first-order derivative spectra of FLM have shown zero-crossing points (ZCPs) at 207.51, 242.34, and 303.30 and KT was determined at the ZCPs 303.30 nm of FLM (Maxima). The first-order derivative spectra of KT have shown zero-crossing points at 220.42, 248.35, 271.47, 321.02, and 394.89 and FLM was determined at the zero-crossing point 220.42 nm of KT (Maxima).
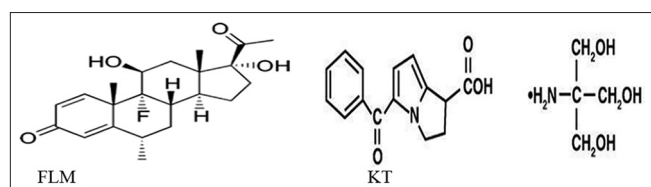
The individual zero-order absorption spectra of FLM and KT in phosphate buffer (pH 4.0) (Method D) were converted into the first-order derivative spectra with the help of inbuilt software. The overlay first-order derivative spectrum of FLM and KT in sodium acetate buffer was shown in Figure 3b. The first-order derivative spectra of FLM have shown zero-crossing points at 241.62 and 310.75 nm, and therefore, KT was determined at the
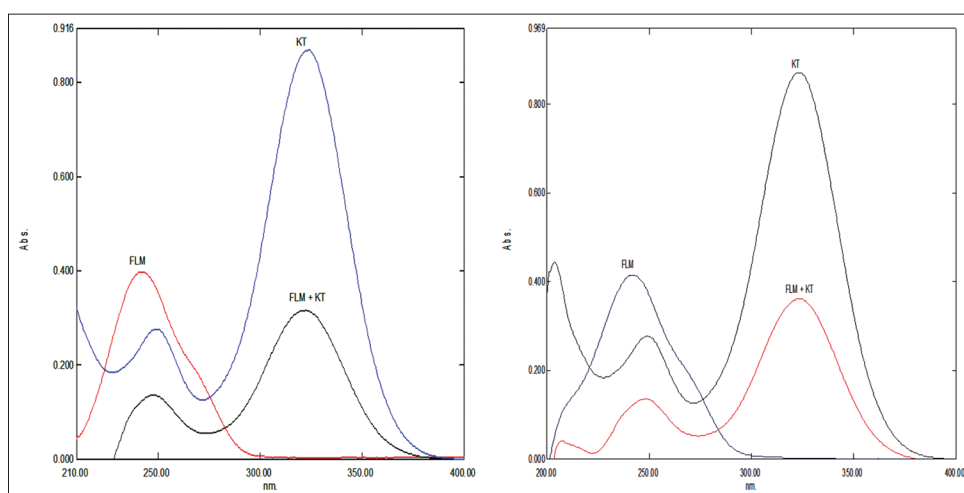

Figure 2: Overlay spectra of fluorometholone (FLM) (10 µg/ml) and ketorolac tromethamine (KT) (10 µg/ml) and formulation (Eye drops) (FLM: KT = 1: 5)
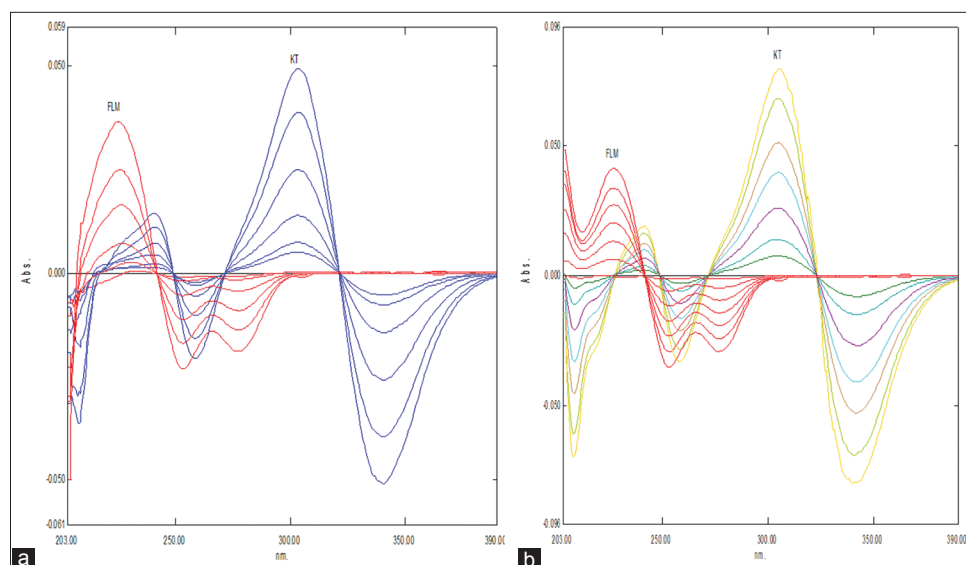
Figure 3: Overlay first-order derivative spectra of fluorometholone and ketorolac tromethamine in (a) sodium acetate (pH 4.0) (b) phosphate buffer (pH 4.0)

zero-crossing point 310.75 nm of FLM (Maxima). The first-order derivative spectra of KT have shown zero-crossing points at 227.84, 249.10, 271.58, and 323.05 nm and FLM was determined at the zero-crossing point 227.84 nm of KT (Maxima).

For Method C and Method D, derivative absorbance (Maxima) of the drug observed at the other drug's zero-crossing point was plotted against the concentration of the drug in the construction of the calibration curve. The individual concentrations of the drugs were determined with the help of calibration curves so plotted.

Precision and accuracy studies

The precision studies were performed at 10 µg/mL and accuracy studies were carried out by standard addition method (80%, 100%, and 120%) and the percentage recovery was calculated.

Assay of FLM and KT

The Eyetrust (Neiss labs Ltd., Mumbai) eye drops containing FLM 0.1% and KT 0.5% were procured from the local pharmacy store, extracted with methanol, and assayed after dilution with sodium acetate and phosphate buffer (pH 4.0) for Method A, B, C, and D.

RESULTS AND DISCUSSION

Two new spectrophotometric techniques - simultaneous equation method (Method A and Method B) and simultaneous derivative method (Method C and Method D) were proposed for the simultaneous determination of FLM and KT in eye

drops using sodium acetate buffer (pH 4.0) and phosphate buffer (pH 4.0).

Method validation

Linearity

Simultaneous equation method
From the overlay zero-order absorption spectra of FLM and KT in sodium acetate buffer and phosphate buffer pH 4.0, the absorptivity values were calculated and substituted in the simultaneous equation given below.

Method A (Sodium acetate buffer pH 4.0)

At 242.13 nm, $A_1 = 422.59\,C_{FLM} + 254.35\,C_{KT}$
At 322.56 nm, $A_2 = 16.13\,C_{FLM} + 924.21\,C_{KT}$

A_1 and A_2 represent the combined absorbance of the marketed formulation solution at 242.13 nm and 322.56 nm in sodium acetate buffer solution, respectively; C_{FLM} and C_{KT} are the concentrations of FLM and KT (g/100 ml), respectively.

Absorptivity of FLM at 242.13 nm = 422.59
Absorptivity of FLM at 322.56 nm = 16.13
Absorptivity of KT at 242.13 nm = 254.35
Absorptivity of KT at 322.56 nm = 924.21.

Method B (Phosphate buffer pH 4.0)

At 242.19 nm, $A_1 = 430.16\,C_{FLM} + 258.53\,C_{KT}$
At 322.23 nm, $A_2 = 360.29\,C_{FLM} + 862.62\,C_{KT}$

A_1 and A_2 represent the combined absorbance of the marketed formulation solution at 242.19 nm and 322.23

nm in phosphate buffer solution, respectively; C_{FLM} and C_{KT} are the concentrations of FLM and KT (g/100 ml), respectively.

Absorptivity of FLM at 242.19 nm = 430.16
Absorptivity of FLM at 322.23 nm = 360.29
Absorptivity of KT at 242.19 nm = 258.53
Absorptivity of KT at 322.23 nm = 862.62.

FLM and KT obey Beer–Lambert's law 1–20 µg/mL in Method A and 2–30 µg/mL in Method B and the linear regression equations were calculated at 242.13 nm and 322.56 nm for both FLM and KT in sodium acetate buffer for Method A [Figure 4] and in phosphate buffer Method B [Figure 5].

Simultaneous first derivative method (D₁)

In the first derivative method (D_1), FLM and KT have shown linearity 1–20 µg/mL in sodium acetate buffer (Method C) and 2–30 µg/mL phosphate buffer (pH 4.0) (Method D). The linearity values were shown in Table 1. Calibration curves were drawn by taking the drug concentration (FLM or KT) on the x-axis and the corresponding derivative absorbance (Maxima) on the y-axis and a straight line graph was obtained in sodium acetate buffer [Figure 6] and in phosphate buffer [Figure 7].

Precision and accuracy studies

The percentage RSD in precision was found to be 0.82–0.97 for FLM and 0.91–1.03 for KT in Method A, B, C, and D which is <2 indicating that the methods are precise and the percentage RSD in accuracy studies was found to be 0.73–1.21 for FLM and 0.69–1.42 for KT in Method A, B, C, and D which is also <2 showing that the methods are accurate.

Assay of FLM and KT

Eye drops preparation available in the medical store was procured and extracted with methanol, centrifuged, and filtered. The filtrate was diluted with sodium acetate buffer
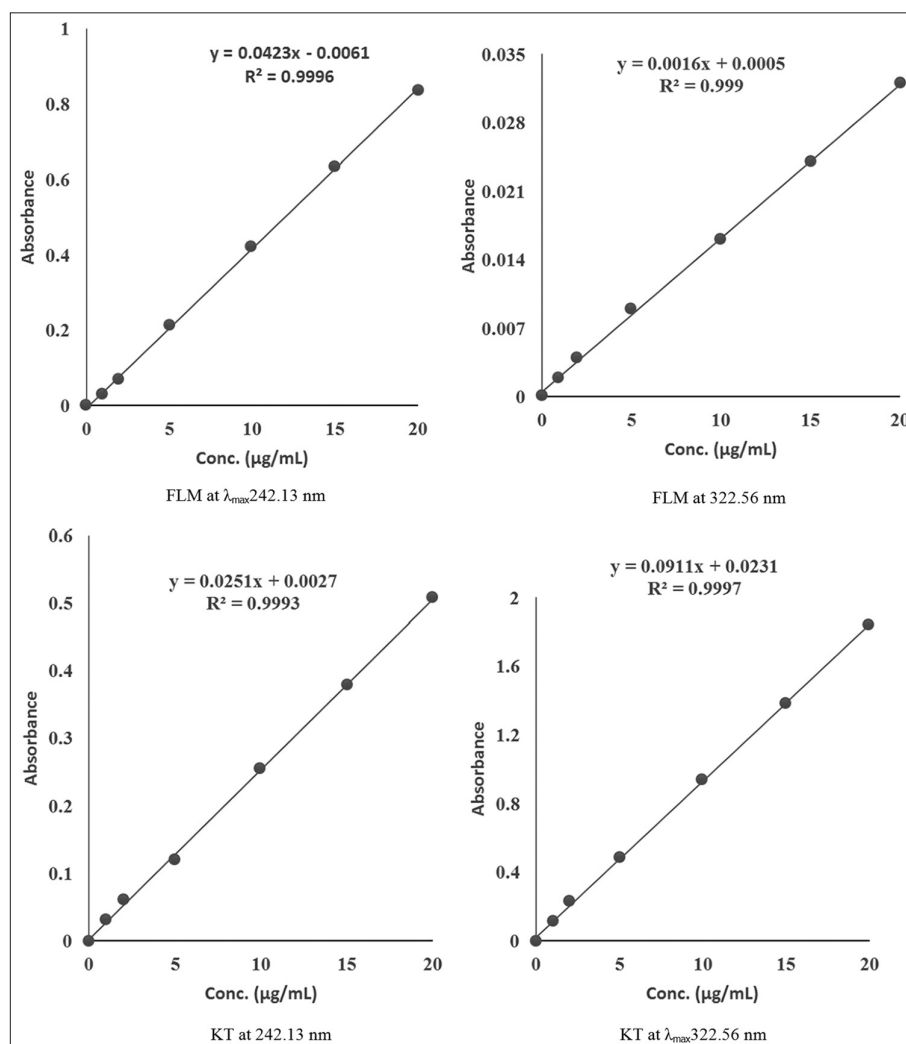

Figure 4: Calibration curves of fluorometholone and ketorolac tromethamine (Method A)
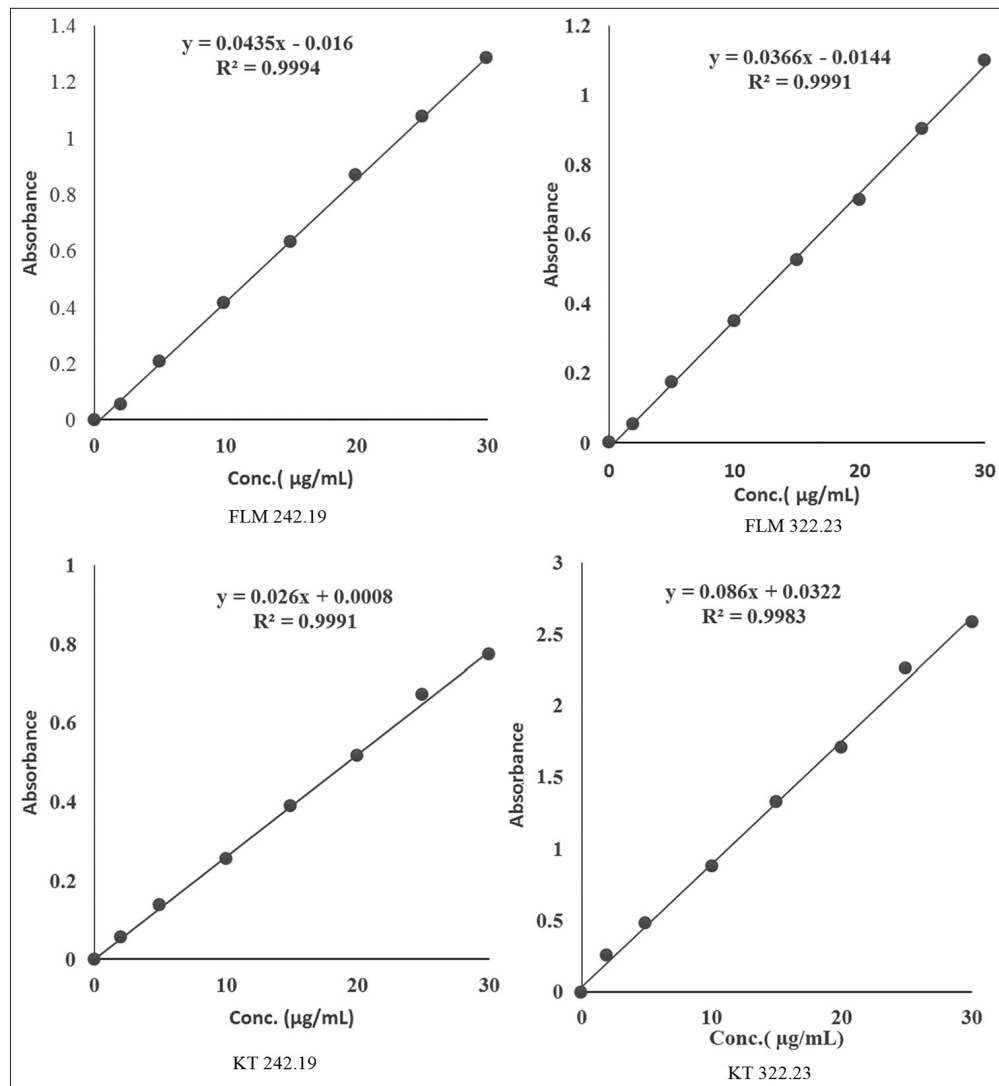
$y = 0.0435x - 0.016$
$R^2 = 0.9994$

FLM 242.19

$y = 0.0366x - 0.0144$
$R^2 = 0.9991$

FLM 322.23

$y = 0.026x + 0.0008$
$R^2 = 0.9991$

KT 242.19

$y = 0.086x + 0.0322$
$R^2 = 0.9983$

KT 322.23

Figure 5: Calibration curves of fluorometholone and ketorolac tromethamine (Method B)

$y = 0.0014x - 0.0003$
$R^2 = 0.9994$

FLM at 227.84 nm (ZCP of KT)

$y = 0.0026x + 0.0008$
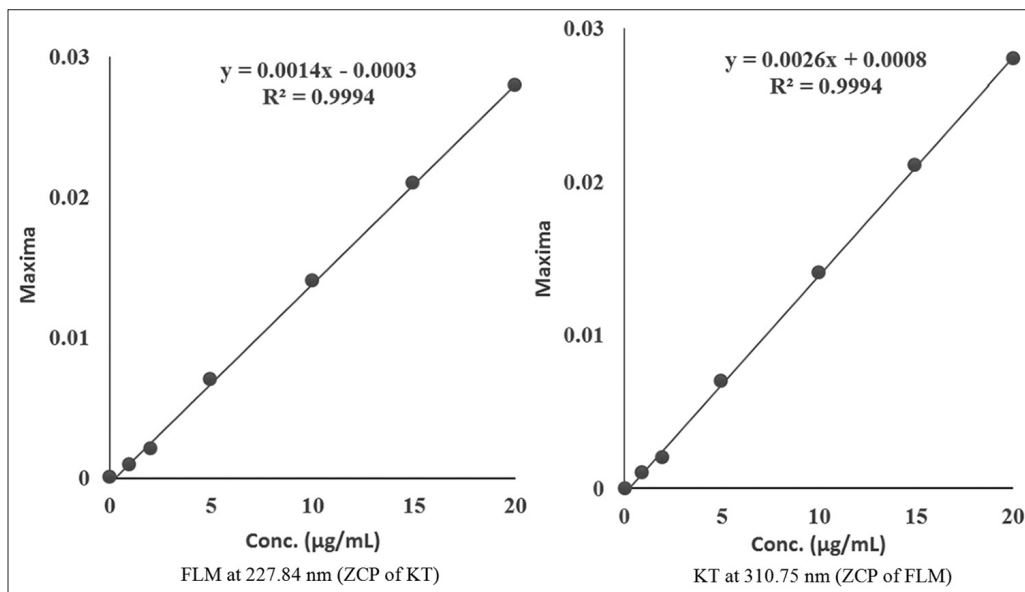$R^2 = 0.9994$

KT at 310.75 nm (ZCP of FLM)

Figure 6: Calibration curves of fluorometholone and ketorolac tromethamine (Method C)

Figure 7: Calibration curves of fluorometholone and ketorolac tromethamine (Method D)
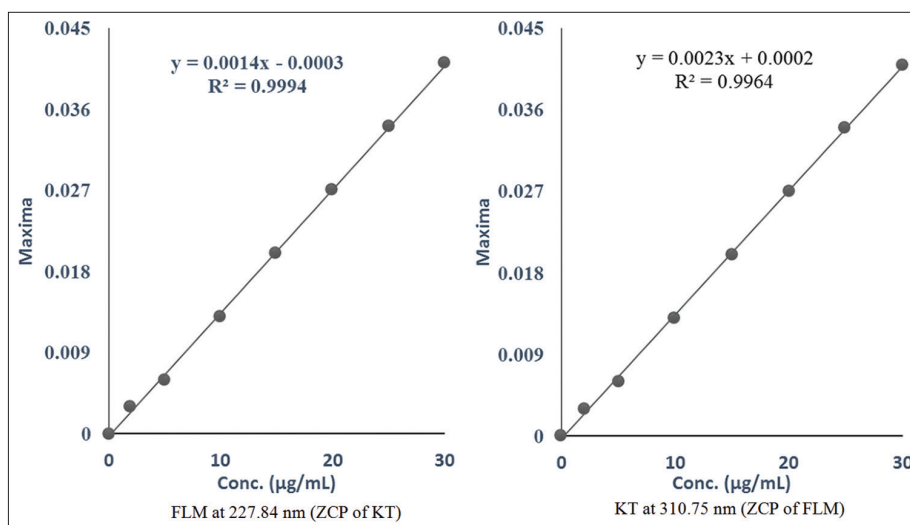
Table 1: Linearity of FLM and KT					
Conc. (µg/mL)		Maxima (Method C)		Maxima (Method D)	
		220.42 nm	303.30 nm	227.84 nm	310.75 nm
FLM	KT	FLM	KT	FLM	KT
1	1	0.001	0.004	-	-
2	2	0.002	0.006	0.003	0.007
5	5	0.007	0.014	0.006	0.012
10	10	0.014	0.026	0.013	0.022
15	15	0.021	0.039	0.020	0.034
20	20	0.028	0.052	0.027	0.044
25	-	-	-	0.034	0.059
30	-	-	-	0.041	0.071

FLM: Fluorometholone, KT: Ketorolac tromethamine

Table 2: Assay of FLM and KT										
Formulation brand	Drug	Label claim (%)	Method							
			*Amount found (%)				*% recovery			
			A	B	C	D	A	B	C	D
Brand I	KT	0.5	0.481	0.491	0.493	0.494	96.2	98.2	98.6	98.8
	FLM	0.1	0.099	0.097	0.098	0.098	99.0	97.0	98.0	98.0

*Mean of three replicates

and phosphate buffer and analyzed with the above two techniques. The percentage recovery obtained in the assay studies was shown in Table 2.

CONCLUSION

The spectrophotometric techniques are very simple, economical, precise, and accurate and therefore can be used successfully for the simultaneous estimation of Fluorometholone and Ketorolac in ophthalmic preparations.

ACKNOWLEDGMENT

REFERENCES

1. Budavari S. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biological. 14th ed. Whitehouse

Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc.; 2006.

2. Shah JA, Maheshwari DG. Development and validation of first order derivative UV spectrophotometric method for simultaneous estimation of fluorometholone acetate and ketorolac tromethamine in ophthalmic dosage form. Indian J Drugs 2014;2:56-64.

3. Annapurna MM, Sevyatha VS, Sushmitha M. Simultaneous determination of ketorolac tromethamine and fluorometholone in eye drops by spectrophotometry. Res J Pharm Tech 2017;10:1179-83.

4. Priti SC, Rajesh RP, Dushyant AS. Development and validation of RP-HPLC method for simultaneous estimation of ketorolac tromethamine and fluorometholone in ophthalmic dosage form. Invent Rapid Pharm Anal Qual Assur 2014;14:15.

5. Ravi JP, Payal PC, Samir KS. Quantification of ketorolac and fluorometholone by RP-HPLC method in ophthalmic formulation. Invent Raopid Pharm Anal Qual Assur Invent 2013;14: 1389.

6. Sharma MV, Patel ND, Bhavesh P, Shah SK. Development and validation of a stability indicating RP-HPLC method for estimation of ketorolac and fluorometholone acetate in ophthalmic formulations. Int J Pharm Nanotech 2014;5:3815-26.

7. Annapurna MM, Narendra A, Sevyatha VS. A new liquid chromatographic method for the simultaneous determination of ketorolac tromethamine and fluorometholone in the presence of hydrochlorothiazide. Int J Green Pharm 2018;12:S220-4.

8. ICH Validation of Analytical Procedures: Text and Methodology, Q2 (R1), International Conference on Harmonization; 2005.