Research Article

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF FLUOROMETHOLONE ACETATE AND KETOROLAC TROMETHAMINE IN OPHTHALMIC DOSAGE FORM

Shah J.A.*, Maheshwari D.G.

**L. J. Institute of Pharmacy, Sarkhej Circle, S. G. Road, Ahmedabad- 382210, Gujarat.**

*For Correspondence:
L. J. Institute of Pharmacy, Sarkhej Circle, S. G. Road, Ahmedabad- 382210, Gujarat.

Access this article online

Website:
www.drugresearch.in

Quick Response Code:

ABSTRACT

A simple, accurate and precise First order derivative spectrophotometric method developed for the simultaneous estimation of Fluorometholone acetate (FLU) and Ketorolac tromethamine (KTC) in ophthalmic dosage form. The first derivative values were measured at 296nm for KTC and 226nm for FLU. The linearity range for zero order was carried out by using the concentration range 10-70µg/ml for both the drug. The correlation coefficient of FLU and KTC for zero order was found to be 0.999 and 0.998 respectively. At zero crossing point (ZCP) of KTC (296nm) FLU showed a measurable derivative absorbance whereas at zero crossing point (ZCP) of FLU (226nm) KTC showed an appreciable derivative absorbance value. Precision study showed that % RSD was within range of acceptable limits (<2%). The % recovery for FLU and KTC was found to be within range of 98-102% and 98-101%respectively. The percentage assay was found to be 98.24% and 99.18% for FLU and KTC. The result of analysis has been validated as per ICH Q2 (R1) guideline.

KEY WORDS: Fluorometholone acetate (FLU), Ketorolac tromethamine (KTC), UV Spectrophotometry, First order derivative Spectrophotometry.

INTRODUCTION

Fluorometholone acetate [9-fluoro-11β,-17-dihydroxy-6α-methylpregna-1, 4-diene-3, 20-dione, 17-acetate] is a Glucocorticoid, is selective phospholipase A_2 inhibitory proteins antagonist and used in Palpebral and Bulbar conjunctiva, cornea and anterior segment of the globe. Fluorometholone acetate is official in USP (USP, 2004). Ketorolac tromethamine [5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid with 2- amino-2-(hydroxymethyl) propane – 1, 3- diol] is a Non steroidal anti-inflammatory drug a selective COX-1 and COX-2 antagonist and used as analgesic and anti-inflammatory agent. Ketorolac tromethamine is official in IP and USP (Indian Pharmacopoiea-10).

Both drugs are formulated together in the form of ophthalmic solution for treatment of allergic conjunctivitis and vernal keratoconjunctivitis (Sweetman SC. 2005, Irvine S.R 1953). The chemical structures of both drugs (The Merk index) were shown in figure 1.
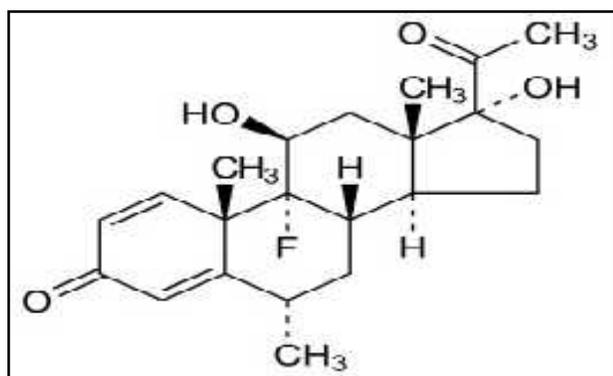
(a)
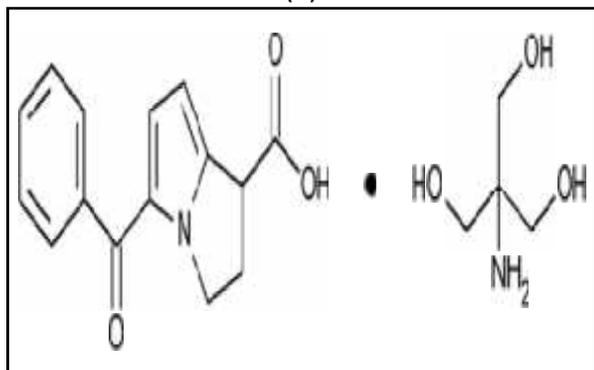
(b)

Figure 1: Chemical structure of Fluorometholone acetate (a) and Ketorolac tromethamine (b).

From literature survey it reveals that very few methods had been developed for determination of Fluorometholone acetate and Ketorolac tromethamine by spectrophotometry and HPLC either alone or in combination with other drug, but no method has been developed for these in combined dosage form by first order derivative UV spectrophotometry. So the purpose of this work was to develop a simple, accurate and sensitive first order derivative spectrophotometric method for determination of Fluorometholone acetate and Ketorolac tromethamine in ophthalmic dosage form.

MATERIALS AND METHODS

Instrument:

The instrument was double beam UV-visible spectrophotometer (Shimadzu, model 1800, Software: UVProbe 2.31) having two matched quartz cells with 1 cm path length. Sonication of sample solutions was done using ultrasonic cleaner (Sonica 2200MH).

Materials:

Fluorometholone acetate (FLU) drug sample was procured from Syntho Pharmaceuticals Pvt. Ltd. Lucknow (Uttar Pradesh, India) and Ketorolac tromethamine (KTC) drug sample was gifted by Cadila Pharmaceuticals Pvt Ltd. Dholka (Gujarat, India).

Eyedrops (Eyetrust, Qualitron Bio Medica Pvt. Ltd. Mumbai, India) was purchased from local market, containing Fluorometholone acetate 0.1% w/v and Ketorolac tromethamine 0.5% w/v per 5 ml Eyedrops. Methanol (95%) was purchase from Astron chemicals Pvt, Ltd. India.

METHODS

Preparation of standard stock solution:

The stock solution having 1000µg/ml concentration of FLU and KTC were prepared separately by dissolving accurately weighed 100mg of both drugs in 100 ml methanol. Further dilutions of standard stock solutions of both drugs were made with methanol to get the working standard stock solutions of 100µg/ml concentration of FLU and KTC.

METHOD DEVELOPMENT (First order derivative)

Selection of scanning range and sampling wavelength:

The standard solutions of FLU and KTC were diluted with methanol individually to get the concentration of 10µg/ml and 50µg/ml respectively and were scanned in UV range 200-400 nm. The λmax of both the drugs were found to be 239nm and 319nm respectively in normal UV spectra shown in figure 2.

Development of first order derivative spectra:

The spectral data was then processed to obtain first order derivative spectrum at wavelength interval of 2nm for the range of 200-400nm. It was observed that FLU shows ZCP at 296nm and KTC shows ZCP at 226nm. At ZCP of FLU (296nm), KTC showed a measurable dA/dλ (Skoog et al. (2000), Beckett et al. (2001)) whereas at ZCP of KTC (226nm), FLU

showed a measurable dA/dλ. Hence the wavelengths 226nm and 296nm were selected as analytical wavelengths for determination of FLU and KTC first order derivative method respectively shown in figure 2.

METHOD VALIDATION

The above proposed method was validated according to ICH Q2 R1 guidelines for validation of analytical procedures (ICH. 2005) in order to determine the linearity, Accuracy, Precision and Assay of marketed formulation.

Linearity and Range:

Calibration curve constructed was linear over a selected range of 10-70µg/ml for both drugs. The aliquots of both the drugs used in linearity studies were converted to first derivative spectra and the derivative absorbance at 296nm and 226nm for FLU and KTC were measured respectively. The calibration curve of responses against concentration was plotted was shown in figure 3 and 4. Each concentration was repeated five times. Correlation coefficient and regression line equations for FLU and KTC were calculated and were shown in table no.1.

Accuracy:

The accuracy of the developed method was determined by finding out the amount of recovery of Fluorometholone acetate and Ketorolac tromethamine. For the accuracy standard addition method was used where, as known amount of FLU and KTC were added to the known concentration (30µg/ml) of Eyedrop solution. The amount recovered was found by measuring the absorbance of the solution and was expressed as mean recovery of samples with upper and lower limits of percent relatives of standard deviation. Recovery was done at three different levels i.e. 80%, 100% and 120%, within the linearity range of both the drugs.

Precision

Repeatability (n=6):

For the repeatability study, from the working stock solution of both drugs, aliquot of 4 ml was transferred to a separate 10 ml volumetric flask and diluted upto mark with methanol such that it gives the concentration of 40 µg/ml of FLU and KTC both. The absorbance of the solutions was measured at 226nm and 296nm respectively. The procedure was repeated six times and % RSD was calculated and shown in table no. 3.

Intraday Precision (n=3):

From the working stock solution, aliquots of 3 ml, 4 ml and 5 ml were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 30, 40 and 50µg/ml for both drugs. The solutions were analysed three times on the same day and % RSD was calculated and shown in table no. 3.

Interday Precision (n=3):

From the working stock solution, aliquots of 3 ml, 4 ml and 5 ml were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 30, 40 and 50µg/ml for both drugs. The solutions were analysed three times on three different days and % RSD was calculated and were shown in table no. 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of detection (LOD) is the minimum concentration of the analyte in the sample which can be analysed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified. The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using following formula. The values of LOD and LOQ for FLU and KTC were shown in table no. 5.

LOD = 3.3 × (SD/Slope)

And LOQ = 10 × (SD/Slope)

Where,SD = Standard deviation of the Y-intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Assay of ophthalmic formulation:

Commercially available marketed ophthalmic solution containing both Fluorometholone acetate and Ketorolac tromethamine(Eyetrust)

were used for the study. Eyedrop solution equivalent to 100 mg of Ketorolac tromethamine and 20 mg of Fluorometholone acetate and 80 mg pure Fluorometholone acetate was added by standard addition method transferred in to a 100 ml volumetric flask to bring both drugs in 1:1 ratio and stock solution of this was prepared in methanol, sonicated for 15 min, the volume was adjusted up to the mark with same solvent. Then solution was filtered through whatmman filter paper No. 41. This stock solution contains Ketorolac tromethamine 100µg/ml and Fluorometholone acetate 100µg/ml. Then the appropriate dilution of 35µg/ml was made using methanol as solvent. All the determinations were carried out in triplicate. The absorbance of the prepared solutions was measured at ZCP of FLU and ZCP of KTC and then the concentration of both the drug was calculated using calibration curve equation. The amount of the drug found in dosage form was shown in table no. 4.

RESULT

(a)   (b)
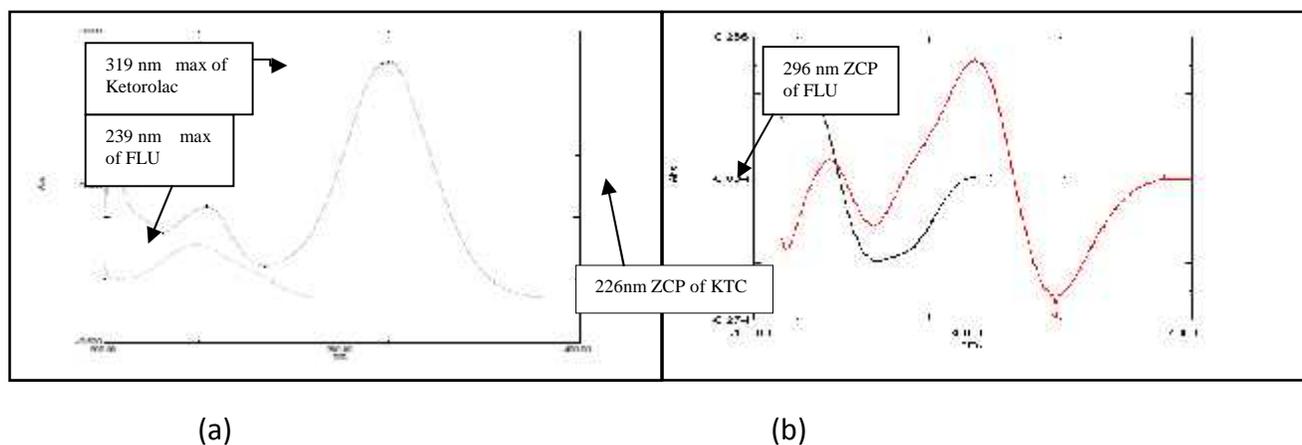
Figure 2: Zero order (a) and first order (b) spectra of FLU (10µg/ml) and KTC (50µg/ml) in methanol
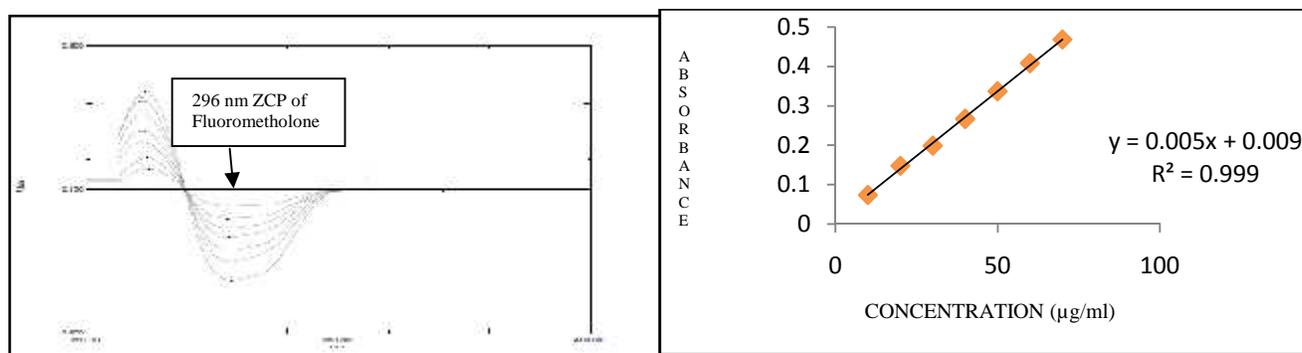
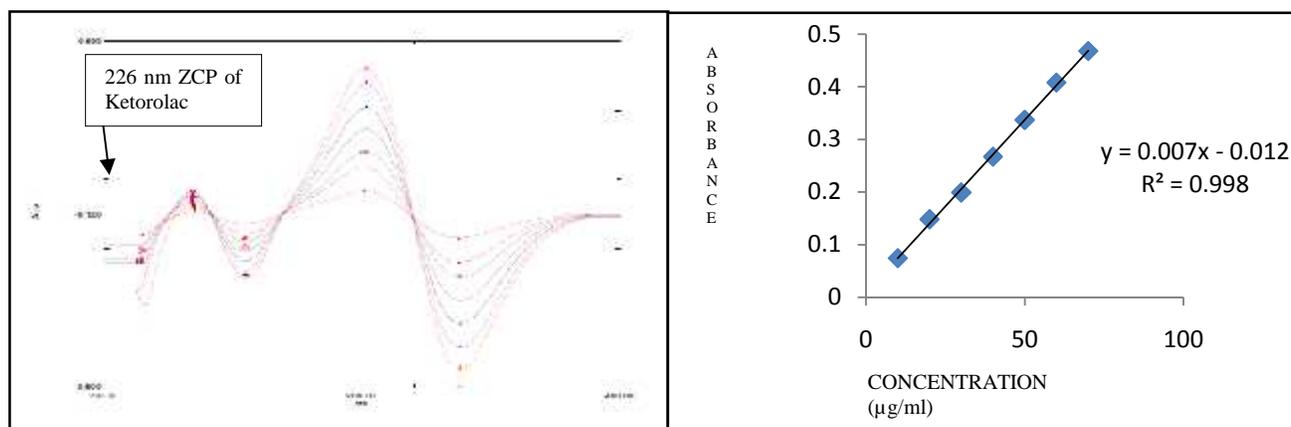**Figure 3: Linearity graph for first order derivative of FLU**

Figure 4: Linearity graph for first order derivative of KTC

Table no. 1: Optical Characteristics

Parameters	Fluorometholone acetate 226 nm	Ketorolac tromethamine 296 nm
Beer's law limit (µg/ml)	10 - 70	10 – 70
Regression equation	Y = 0.005x + 0.009	Y = 0.007x – 0.012
Slope (m)	0.005	0.007
Intercept (c)	0.009	0.012
Correlation coefficient (R^2)	0.999	0.998

Table no. 2: Results of Recovery studies

Drug	Concentration of STD drug	Recovery level (%)	Amount of drug added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery ± SD
FLU	30 µg	80	24	53.46	98.80 ± 0.0049
		100	30	59.41	99.01± 0.0008

		120	36	66.52	100.7 ± 0.0009
KTC	30 µg	80	24	54.22	100.4 ± 0.0012
		100	30	59.90	99.84 ± 0.0009
		120	36	66.42	100.6 ± 0.0009

*SD = standard deviation, *STD = standard

Table no. 3: Repeatability, Inter-day and Intra-day precision of Fluorometholone and Ketorolac

Drug	Concentration (µg/ml)	Average ABS ± SD	% RSD
REPEATABILITY (n=3)			
FLU	40	0.231 ± 0.0033	1.471
KTC	40	0.268 ± 0.0009	0.354
INTRA-DAY PRECISION (n=6)			
FLU	30	0.184 ± 0.0037	1.801
	40	0.233 ± 0.0028	1.211
	50	0.291 ± 0.0023	0.809
KTC	30	0.199 ± 0.0042	1.781
	40	0.268 ± 0.0012	0.464
	50	0.338 ± 0.0017	0.501
INTER-DAY PRECISION (n=6)			
	30	0.181 ± 0.0009	0.519

FLU	40	0.230 ± 0.0033	1.475
	50	0.288 ± 0.0033	1.143
KTC	30	0.199 ± 0.0042	1.781
	40	0.268 ± 0.0004	0.175
	50	0.340 ± 0.0026	0.635

*SD = standard deviation, ABS = Absorbance

Table no. 4: Analysis of Ophthalmic formulation

Drug	Label claim (mg/ml)	Amount found (mg/ml)	% Drug found ± SD
FLU	1	0.982	98.24 ± 0.0009
KTC	5	4.960	99.18 ± 0.0047

*SD = standard deviation

Table no. 5: Limit of detection (LOD) and Limit of Quantification (LOQ)

Parameters	Fluorometholone acetate	Ketorolac tromethamine
LOD (μg/ml)	0.818	0.584
LOQ (μg/ml)	2.481	1.771

DISCUSSION

The present paper describes the estimation of FLU and KTC in ophthalmic dosage form by First order derivative method. The Beer- Lambert's concentration range was found to be 10-70μg/ml for both drug FLU and KTC at 226 nm and 296 nm respectively. The correlation coefficient was found to be

0.999 for FLU and 0.998 for KTC (Table no. 1) for proposed method. Precision was determined by studying repeatability, intraday and interday precision. The standard deviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision(Table no. 3). The values of LOD and LOQ were 0.818μg/ml and 2.481μg/ml for FLU and 0.584μg/ml and 1.771μg/ml for KTC respectively (Table no. 5). Percentage estimation of FLU and KTC in ophthalmic dosage form was 98.24% and 99.18% by

proposed method respectively (Table no. 4).

CONCLUSION

A simple, accurate and precise UV first order derivative spectrophotometric method has been developed for the estimation of FLU and KTC in combination. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by selecting zero crossing point in the derivative spectra of each drug at selected wavelength. The developed method can be successfully applied to analysis of marketed formulation.

REFERENCES

1. United State Pharmacopoeia. (2004). USP 27, NF 22, USP Convention INC. Rockville, Asian edition. 2004, 818- 819, 1058- 1060.

2. Indian Pharmacopiea-2010, Government of India Ministry of Health and Family welfare, Published by Indian Pharmacopeia Commission, New Delhi, Vol. 3; 1543-1546.

3. Sweetman, S.C. (2005). Martindale: The Complete Drug Reference. Pharmaceutical Press, London.

4. Irvine, SR. (1953). A newly defined vitreous syndrome following cataract surgery. Am J Ophthalmol. 36(5), 599-619.

5. An encyclopedia of chemical, drugs and biological. The Merk index. 14th edition. Published by Merk research laboratory. 714, 1056.

6. Drug bank, "Fluorometholone", November 2013, www.drugbank.ca/drugs/DB00324.

7. Drug information online "Ketorolac tromethamine" November 2013, www.drugs.com/ppa/ketorolac-tromethamine.html.

8. Skoog; et al (2001). Principles of Instrumental Analysis. 6th edition. Belmont, CA: Thomson Brooks/Cole publisher. 169–173.

9. Beckett, A.H. and Stenlake, J.B. (2001) Practical Pharmaceutical chemistry. 4th edition. CBS publishers and distributors. 296-300.

10. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use on Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1), 2005.

11. Teja, S.G. Venugopal, V. Rao, PLKM. K Kavitha. (2011). Revalidation and analytical evaluation of Ketorolac tromethamine by HPTLC using reflectance scanning densitometry, International journal of research in pharmacy and chemistry. 1(2): 129-132.

12. Altuntas, T.G. Korkmaz. F. Nebioglu, D. (2000). Determination of Tetrahydrozoline hydrochloride and fluorometholone in pharmaceutical

formulations by HPLC and derivative UV spectrophotometry. Pharmazie. 55(1): 49-52.

13. Tsvetkova, B.G. Pencheva, I.P. Peikov, P.T. (2012). HPLC determination of Ketorolac tromethamine in tablet dosage forms, Pelagia Research Library. 3 (4): 400-403.

14. Gandla, K. Kumar, J.M.R. Bhikshapathi, D.V.R.N. Gajjela, V. Spandana, R. (2012). A validated RP-HPLC method for simultaneous estimation of Febuxostat and Ketorolac tromethamine in pharmaceutical formulations, Journalof drug delivery and therapeutics. 2(3): 173-176.

15. Vichare, V. Tambe, V. Joshi, S.V. Dhole, S.N. (2013). New Simultaneous UV-Visible Spectrophotometric Methods for Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form, Asian journal of pharmaceutical analysis. 3(2): 53-57.