Research Article

A Validated UV Spectrophotometric Method for the Estimation of Olopatadine and Ketorolac Tromethamine in Ophthalmic Dosage Form

Yatri.J.Bhatt[1], Sandip.K.Sharma[1], Parmeshwari.J.Multani[1*]
[1]Saraswati Institute of Pharmaceutical Sciences, Gandhinagar, Gujarat, India.
***Corresponding author's E-mail:** parmeshwari_halen@rediffmail.com

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ABSTRACT

To develop simple and economical UV spectrophotometric method for the estimation of Olopatadine and Ketorolac tromethamine in the combined ophthalmic dosage form available in the market for conjunctivitis. Two drugs Olopatadine and Ketorolac tromethamine are present in the ratio of 4:1. The two drugs were simultaneously estimated by first order derivative. From the overlain first order derivative spectra wavelength 340nm (ZCP for Olopatadine) and 229nm (ZCP for Ketorolac) were selected for the quantification of Olopatadine and Ketorolac. The method was validated as per the ICH guidelines and the results were statistically validated. Linearity was observed in concentration range of 8.2-45µg/ml for Olopatadine and 2.5-50µg/ml for Ketorolac. The accuracy of the method was evaluated by recovery studies and good recovery results were obtained between 98% to 100% and the relative standard deviation was found to be below 2% . A simple, accurate, sensitive and economical UV-spectrophotometric method for the estimation of Olopatadine and Ketorolac in combined dosage form has been developed which can be employed in the industry for the routine analysis.

Keywords: Olopatadine (OLO), Ketorolac tromethamine (KETO), First order derivative, Spectrophotometric method.

INTRODUCTION

Olopatadine (OLO),11-[(Z)-3-(Dimethylamino) propylidene] -6-11-dihydrodibenz[b,e] oxepin -2-acetic acid hydrochloride, is widely used as an antihistaminic[1]. Ketorolac(KETO), (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3- propanediol, is an anti-inflammatory drug[2]. The combination of two drugs is used in conjunctivitis. OLO (selective H1 receptor antagonist) inhibits the release of histamine from the mast cells[3] and KETO (non selective COX inhibitor) inhibits prostaglandin synthesis by competitive blocking of COX enzyme[4]. The two drugs alone and in combination with other drugs are reported to be estimated by UV[5-8], HPLC[9] and RP-HPLC[10-13]. The present combination is not official in any pharmacopoeia hence no official method is available. Literature survey does not reveal any UV spectrophotometric method for the estimation of these two drugs from the combined dosage form. In the present work, the 1st order derivative spectrophotometric method has been developed for the estimation of OLO and KETO in combined ophthalmic dosage form. The method was validated as per ICH guidelines.

MATERIALS AND METHOD

Instrument

A double beam Shimadzu UV-Visible spectrophotometer model 1800 shimadzu, loaded with UV probe 2.3 software, with spectral bandwidth of 2nm, wavelength accuracy ±0.5 nm and a pair of 1 cm matched quartz cells was used for spectroscopy.

Material

OLO was obtained as gift sample from Ajanta Pharma Limited, Mumbai and Ketorolac was obtained as a gift sample from Sun Pharmaceutical Ind. Ltd., Baroda. All the other reagents, chemicals and solvents used were of AR grade. The combined dose eye drops (Olopat KT) was purchased from local pharmacy.

Preparation of stock solution

Accurately weighed quantity of OLO (50mg) and KETO (250mg) were transferred to two separate 50ml volumetric flask and dissolved in distilled water and diluted to mark with same solvent. This resulted in stock solution of OLO (1000µg/ml) and KETO (5000µg/ml).

Method

From the stock solution of OLO and KETO aliquots were taken and proper dilution was performed to yield the solution of 8.2-45µg/ml for OLO and 2.5-50µg/ml for KETO. The resulting solution were scanned in UV from 200-400nm, converted to first order derivative. The overlain first order derivative spectra of OLO and KETO revealed ZCP at 340nm and 229nm respectively (Fig:1). The calibration curve was plotted and regression equation computed.

Method validation

The proposed method was validated as per ICH guidelines[14].

Linearity and Range

Linearity was evaluated in the range of 8.2-45µg/ml for OLO and 2.5-50µg/ml for KETO and expressed in the terms of regression coefficient as shown in table 1.

Precision

Precision was determined by analysing three concentrations three times on the same day for intraday precision and on three consecutive days for interday precision. %RSD was calculated as shown in Table 2.

Accuracy

The recovery studies were carried out by standard addition at three different levels (50%, 100% and 150%) for OLO and KETO in triplicate. The solutions were analysed, present recoveries calculated (Table 3).

Assay of ophthalmic formulation

From the ophthalmic formulation (Olopat-KT) 1ml equivalent to 1 mg OLO and 4 mg KETO was pipette out and transferred into 10 ml volumetric flask, dissolved and volume made up to 10 ml with distilled water. Aliquot (1ml) was transferred to 10ml volumetric flask and diluted to volume with distilled water to yield 10µg/ml OLO and 40µg/ml KETO. The solution was scanned from 200-400nm, converted to first order derivative spectrum and absorbance measured at 229nm (OLO) and 340nm (KETO). Results are reported in Table 4.

Table 1: Quantitative parameters of the method

Parameter	OLO(229 nm)	KETO(340 nm)
Linearity (µg/ml)	8.0-45	2.5-50
Regression equation	y=-0.002x-0.002	y=-0.00176x-0.002
Slope	-0.002	-0.00176
Intercept	-0.002	-0.002
R^2 value	0.9947	0.999
LOD (µg/ml)	1.24	1.47
LOQ (µg/ml)	3.77	4.48

RESULTS AND DISCUSSION

The linearity range for OLO and KETO was determined to be 8.0-50µg/ml and 2.5-50µg/ml respectively. The percent recovery for OLO and KETO were found to be 98-99 % and 99.23-99.70% respectively. The reproducibility of the method was confirmed by the %RSD values for intraday and interday which were less than 2. The results of the commercial formulation was in good agreement with the label claim.

Table 2: Precision (Intraday & Interday)

Drug	Concentration (µg/ml)	Intraday		Interday	
		Abs*±SD	%RSD	Abs*±SD	%RSD
OLO	8.0	0.016±0.00047	2.88	0.017±0.00043	2.45
	10	0.026±0.00047	1.79	0.023±0.00047	2.02
	20	0.036±0.00047	1.29	0.037±0.00047	1.26
KETO	35	0.061±0.00094	1.52	0.060±0.00094	1.55
	40	0.072±0.00047	0.65	0.073±0.00094	1.28
	42	0.076±0.00124	1.63	0.075±0.00124	1.65

*n=3, SD-standard deviation, RSD- Relative standard deviation

Table 3: Accuracy (Recovery study of OLO and KETO)

Drug	Level	Amt taken (µg/ml)	Std taken (µg/ml)	Total conc. (µg/ml)	Conc.found* (µg/ml) Mean±SD	% Recovery* ±SD
OLO	50%	10	5	15	14.75± 0.204	98.33± 1.36
	100%	10	15	25	24.83± 0.169	99.33±0.679
	150%	10	25	35	34.8± 0.2160	99.40±0.649
KETO	50%	20	4	24	23.81± 0.062	99.23±0.261
	100%	20	8	28	27.82± 0.074	99.35±0.265
	150%	20	12	32	31.58± 0.168	99.70± 0.523

*mean of three determinations, SD- standad deviation.

Table 4: Result of commercial formulation analysis

Label claim (mg/tablet)	Label claim found* (mg/tablet) ±SD	%Assay±SD
OLO (1mg)	0.98±0.0102	98.83±1.02
KETO (4mg)	3.94±0.0208	98.65±0.52

*mean of three determinations, SD-standard deviation.

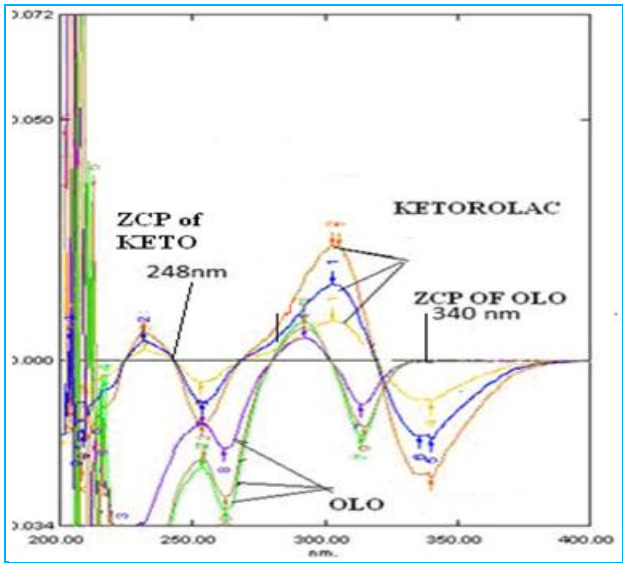

Figure 1: First order derivative spectra of OLO and KETO

CONCLUSION

The proposed method is simple, cost effective, precise and accurate enough for the determination of OLO and KETO in their combined dosage form. The developed method can be successfully applied to routine analysis in OLO and KETO in pharmaceutical formulation.

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