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ABSORPTION RATIO METHOD FOR THE ESTIMATION OF MOXIFLOXACIN HCl & KETOROLAC TROMETHAMINE IN THEIR COMBINED DOSAGE FORM BY UV-VISIBLE SPECTROSCOPY

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ABSTRACT

A simple, precise, accurate and economical method, without any extraction step, for estimation of moxifloxacin.HCl & ketorolac tromethamine in bulk and pharmaceutical products was developed and validated by absorption ratio method. Identification and quantification was carried out using a UV detector, with working wavelength of 322 nm & Isobestic point 305 nm. in water medium(AR grade). The method was validated as per ICH guidelines with respect to its specificity, linearity, range, accuracy and precision in the analytical media. Regression analysis of Beer-Lamberts plots showed good correlation in the concentration range 2-20mcg/ml. Statistical treatment of the results reflects that the proposed method is precise, accurate and easily applicable for the determination of moxifloxacin.HCl in bulk and pharmaceutical preparation.

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Key Words

Moxifloxacin HCl, Ketorolac
tromethamine,UV spectroscopy

INTRODUCTION

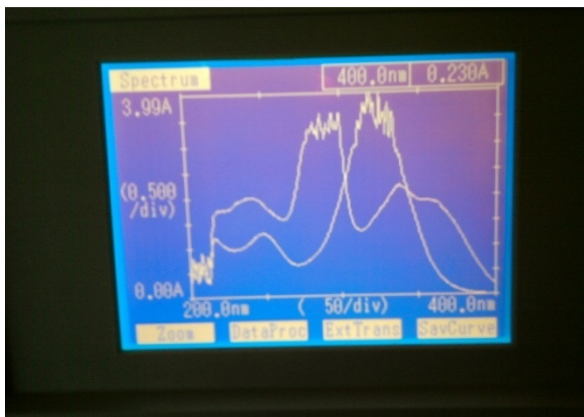


Fig1.- Overlain spectra of MOXI and KETO

Moxifloxacin.HCl (Fig. 1) is a fourth generation fluoroquinolone, the antimicrobial activity of which depends upon inhibition of DNA gyrase (bacterial topoisomerase II), an enzyme necessary for DNA replication, transcription, repair and recombination. Moxifloxacin has *in-vitro* and *in-vivo* activities against wide range of gram+ve and gram-ve bacteria. [1] Ketorolac tromethamine is a potent nonnarcotic analgesic compound with cyclooxygenase inhibitory activity which has been developed for oral and parenteral use.(2) MoxifloxacinHCl (MOXI) is 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS) octahydro-6H- pyrrolo[3,4-b]pyridin-6-yl]-4- oxo-1,4 dihydroquinoline-3- carboxylic acid hydrochloride. Ketorolac tromethamine (KETO) is 1H-Pyrrolizine-1-carboxylic acid, 5benzoyl-2, 3dihydro, with 2 amino-2-(hydroxymethyl)-1, 3- propanediol For routine analysis, a simple and cost effective analytical method is preferred. The objective of the present study was to develop a simple precise, accurate and economic analytical method with better detection range, for the estimation of moxifloxacin HCl & ketorolac tromethamine in bulk and pharmaceutical formulation. In the analytical method developed, water was used as analytical media, as both drug was found to be stable in water , & also water is economic as compare to other media so this method is simple precise, accurate and economic. The developed method was validated as per ICH guidelines [3] and suitable statistical tests were performed on validation data.

MATERIALS AND METHODS

Chemical and Reagents

Moxifloxacin.HCl was obtained as gift sample from Dr.Reddy's laboratories Ltd. Hyderabad, India.& ketorolac tromethamine was obtained as gift sample from Microlab's Pvt Ltd Bangalore, India. Marketed eye drop of containing equivalent to 5mg/ml of both drug. All other chemicals and the reagents used were of Anal AR grade.

INSTRUMENTS

Spectrophotometer : Double beam UV –visible spectrophotometer with 10 mm matched quartz cell, Thermo UV 2401 PC (Japan)

Calibration Standards

Stock solution : The aliquot portions of stock standard solutions of MOXI and KETO were diluted appropriately with solvent to get a series of concentration between 2-20 ($\mu\text{g/ml}$) of MOXI and KETO. Similarly aliquot portions of stock standard solutions were mixed and diluted to get series of concentration between 2-20 ($\mu\text{g/ml}$). The absorbance of each solution was measured at 322 nm and 305 nm in 1 cm cell against solvent blank. The graphs plotted as concentration Vs absorbance at selected wavelengths are shown in Fig. No. 2 to 4

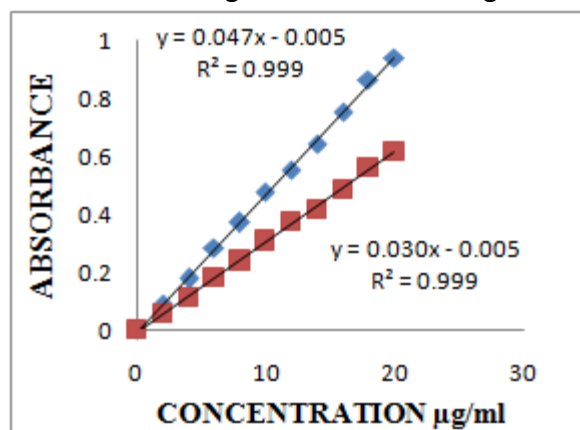


Fig:2 Plot of Beer-Lambert study for KETO

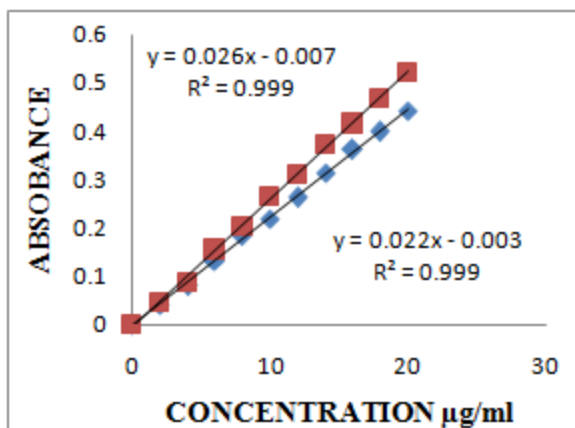


Fig. No.3: Plot of Beer-Lambert study for MOXI

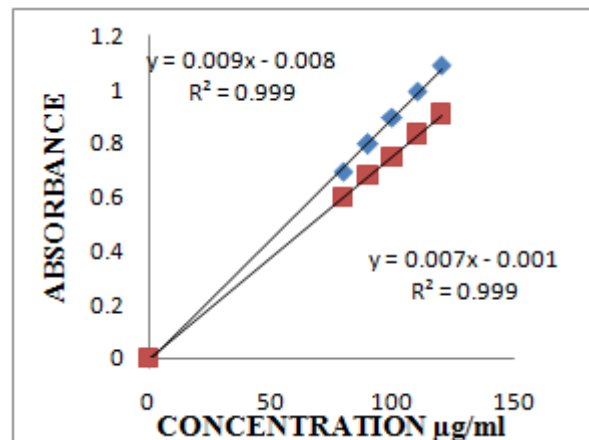


Fig. No. 5: Linearity and range for MOXI and KETO.

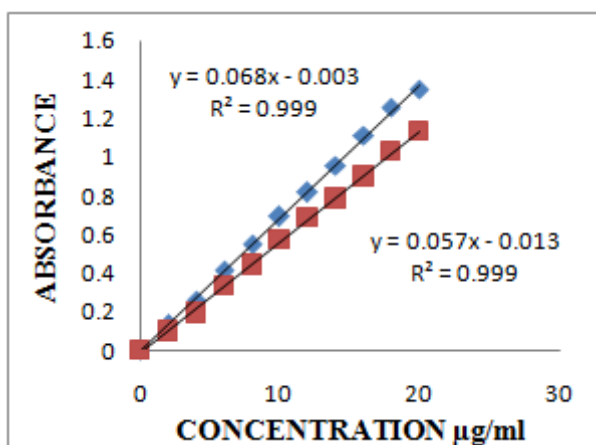


Fig. No.4: Beer-Lambert study of Lab. mix.

Analysis of Laboratory mixture: In order to see the feasibility of proposed method for simultaneous estimation of MOXI and KETO in pharmaceutical formulations, the method was first tried for the estimation of the drugs in standard laboratory mixture data shown in table no.1

Analysis of marketed formulation: The MOXI & KETO solution were prepared of 50 (µg/ml) from marketed formulation. The solution was filtered through Whatman filter paper no. 41. The absorbance of sample solution was measured at 322 nm and 305 nm in 1 cm cell against blank. Data shown in table no.1

Tables no 1: Result of estimation of MOXI or KETO in Laboratory mixture & marketed formulation.

Sr.no.	Sample	Statistical data	% Label claim		% Recovery	
			MOXI	KETO	MOXI	KETO
1.	Standard Laboratory mixture	Mean	100.4	100.87	-	-
		S.D.	1.732	1.457	-	-
		C.V.	1.72	1.444	-	-
2.	Marketed formulation (Mofloren-KT)	Mean	99.56	99.56	99.795	99.785
		S.D.	1.7378	1.737	0.762	0.752
		C.V.	1.74	1.744	0.763	0.753

Recovery studies:

Recovery study was done by standard addition method. Accurately weight the quantity of standard & sample drug. Prepare to it about 50 ($\mu\text{g/ml}$) of solution. The solution was filtered through Whatman filter paper no.

41. The absorbance of sample solution was measured at 322 nm and 305 nm in 1 cm cell against blank.

Accuracy: Accuracy of an analytical method is the closeness of test results obtained by the method to the true value. It was ascertained on the basis of recovery studies performed by standard addition method. Shown in table no 4.

Table No.4: Results of Accuracy studies of MOXI and KETO

Sr. No.	Weight of eye drop $\mu\text{g/ml}$	Amount added $\mu\text{g/ml}$		%Recovered	
		MOXI	KETO	MOXI	KETO
1	50	2	2	99.11	99.31
2		4	4	100.68	99.12
3		6	6	99.15	99.76
Mean				99.64	99.39
\pm S.D.				0.895	0.328
C.V.				0.0089	0.0033

Precision:

Precision of an analytical method is the degree of agreement among individual results when the method is applied repeatedly to multiple readings of a homogeneous sample. It is expressed as S.D. or R.S.D. of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

and absorbance of same sample were recorded on different days.

- **Different analyst** :The sample solutions were prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated

Ruggedness:

The studies of ruggedness were carried out under two different conditions:

- Days and
- Analyst

Intraday:

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The % label claim was calculated.

- **Interday (Different days):** Same procedure was performed as under marketed formulation analysis

Linearity and range:

Accurately weighed quantities of formulation equivalent to 80, 90, 100, 110, 120 % of label claim were taken and dilutions were done appropriately to obtain a concentration in the range of 80-120% of the test concentration and absorbance were recorded at 322 nm and 305 nm. MOXI and KETO were found to be linear in 80% - 120% of the test concentration.

RESULTS AND DISCUSSIONS

MOXI and KETO solution individually follows the Beer-Lambert's law over concentration range 2- 20 ($\mu\text{g/ml}$) at selected wavelength. The mixture of the two drugs also obeys the Beer-Lambert's law over concentration range 2- 20 ($\mu\text{g/ml}$). Both the drugs showed additivity of

absorbance at selected wavelengths. The A (1%, 1cm) values for both the drugs were determined at the selected wavelengths.

The proposed methods applied for the estimation of the drugs in standard laboratory mixture has yielded very encouraging results and thus it was extended for the estimation of drug in marketed ophthalmic formulation. Recovery studies were performed by adding a known amount of standard drug to preanalysed sample and contents were reanalyzed by proposed methods. The summary of this method is shown in the Table No. 1

Validation:

Validation is normally done to assure the reliability of the proposed method and was performed as per the ICH guidelines for the following criteria.

1) Accuracy: Accuracy of method is ascertained by recovery studies performed at different levels of concentrations. Mean % recovery were found to be within 98-102%.

2) Precision: The methods were found to be precise with \pm S.D. of 1.72 and 1.444 for the estimation of MOXI and KETO respectively by Absorption ratio method table no.3

Table No.3: Results of Precision studies of MOXI and KETO

Sr.No.	Statistic data	MOXI	KETO
1	Mean	100.3	100.86
2	\pm S.D.	1.732	1.457
3	C.V.	1.72	1.444

3) Ruggedness:

The methods were found to be rugged with no significant changes on test result upon change of analytical conditions like, different time (Intraday), different day (Interday), and Different analyst. Summary of result for ruggedness study is depicted in the table no. 2

Table No. 2: Summary of result of Ruggedness studies

Parameter	Statistical data	Absorption correction method	
		MOXI	KETO
Interday	Mean	99.56	99.56
	\pm S.D.	1.7378	1.737
	C.V.	0.017	0.017
Intraday	Mean	99.56	99.56
	\pm S.D.	1.7378	1.737
	C.V.	0.017	0.017
Different analyst	Mean	99.43	99.53
	\pm S.D.	0.3511	0.6027
	C.V.	0.3531	0.6055

4) Linearity and range:

The study of linearity and range was performed as per the USP/ICH recommendation. MOXI and KETO marketed formulation was found to be linear in the range of 80% to 120 % of test

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