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The Concurrent Analysis of Moxifloxacin Hydrochloride and Ketorolac Tromethamine in Ocular Drops Using Chemometrics Methodology

CEEMA MATHEW[1]*, DEVARA SRIKANTH[1], SHASHIKALA METRI[2], NEKKANTI SUBASH[1], NAGA SHRAVANI MAHALAKSHMI SRIRAM[1] and SWATHI BALASUBRAMANIAN[1]

[1]Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Hyderabad-Telangana-500090, India.
[2]Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Hyderabad-Telangana-500090, India.
*Corresponding author E-mail: nirujose2003@gmail.com

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ABSTRACT

An ocular dosage form of Moxifloxacin Hydrochloride and Ketorolac Tromethamine is available, and for the coexistent analysis, a UV-based chemometrics method is developed. An economical method that uses very few organic solvents was built based on the mean centering of the ratio spectral data values with a computer Program based on MATPLOTLIB and Python. Both drugs gave linear results across 2-10 μg/ml with good correlation coefficients. The analytical process was subjected to validation for the specified criteria concerning ICH guidelines.

Keywords: Chemometrics, Moxifloxacin Hydrochloride, Ketorolac Tromethamine, Ratio Mean Centering method.

INTRODUCTION

In ocular infections, it's a common practice to use an effective antibiotic in combination with an NSAID or steroid in order to alleviate the symptoms at a faster rate. Moxifloxacin Hydrochloride (MOX) and Ketorolac Tromethamine (KET) is such a combination of an antibiotic and an NSAID used in bacterial eye infections. MOX is chemically1-Cyclopropyl-7-[(1S,6S)-2,8diazabicyclo [4.3.0] nonan-8-yl]-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid salt of a fluoroquinolone antibiotic[1]. KET is chemically 2-amino-2-(hydroxymethyl) propane-1,3-dio5-benzoyl-2,3-dihydro-1H-pyorrolizine-1-

carboxylic acid. It is a synthetic tromethamine salt of ketorolac, possessing analgesic, antipyretic, and anti-inflammatory activities[2]. The drug structures of MOX & KET are presented in Figure. 1.

MOX blocks DNA replication in bacteria by binding to the bacterial enzymes, topoisomerase II and IV. Ketorolac blocks COX-1 and COX-2, hence it is non-selective towards the COX enzyme[3]. The effects of MOX and KET alone, in fixed-dose combinations, and conjunction with other medications were investigated using various methods. Moxifloxacin has been investigated using UV spectrophotometric methods[4-10], HPLC[11-15], and HPTLC[16] either alone or in combination with other medications. The combined use of MOX and KET was investigated using UV Spectrophotometry[17-19] and HPLC[20-23]. While several analytical techniques were described for single medications as well as different combinations, there were fewer published techniques for the coexistent estimation of KET and MOX in combination. Hence, considering this, we thoughtfully planned the present investigation to develop a green method using chemometrics based on ratio mean centering (RMC) for the concurrent analysis of the drugs. Using statistical or mathematical tools, one can retrieve useful information from chemometrics methods to help with the QC analysis.

MATERIALS AND METHODS

Instruments and Chemicals

Shimadzu UV-Visible spectrophotometer was employed for the spectral recording. Python and Matplotlib-based software programs assisted the ratio mean centring of the data of the ratio spectrum. MOX and KET freebies from Hetero Drugs Ltd, Hyderabad, India, were used.

Process outline

Normal spectra of MOX were divided by a KET spectrum of a suitable concentration, and to quantify KET, its spectra were divided by an appropriate concentration of MOX spectrum. The ratio spectra were mean-centred using the Program, and the standard graph was plotted with the measured amplitudes Vs concentration from the ratio mean-centred spectrum. An economical method that utilizes no organic solvent has been developed.

Method Validation

The method validation strategy is conducted as outlined below and is in accordance with ICH guidelines[24]**.** For testing Linearity, 10 mg of each medication was used to prepare stock solutions and further dilutions of MOX and KET, each separately, in a range of strengths from 2 to 10 μg/ml using 0.1 M urea. The stored UV spectrum of each drug, derived by the scanning process were divided the spectrum of the other drug; KET spectrum of ten μg/ml was the divisor for MOX spectrum, and six μg/ml of MOX spectrum was the divisor for KET spectrum. The collected data sets of the ratio spectrum were mean centered using the written software program, explicitly designed for this purpose. The reliability curves were developed by charting the amplitudes against the respective concentrations[25-26]**.**

The accuracy was evaluated using the standard addition technique by comparing the measured value to the actual amount. Three levels of spikes with standards (80%, 100%, and 120%) were applied to commercially available eye drops. The RMC method was verified by estimating the amount of pharmaceuticals experimentally versus the theoretical amount for each spiking. The responses noted at 284 nm and 314 nm were used to determine the amounts of MOX & KET, respectively, while the spiking and subsequent readings were duplicated three times[25-26]**.**

The precision evaluation calculates how closely test results match when a sample is read multiple times. The analysis of three standard solutions of MOX & KET of the same strength (2, 6, and 10 μg/ml) was repeated to ensure repeatability in the results on a single day and three different days for intra-day and inter-day precision, respectively[25-26]**.** The success index criteria for accuracy and precision are to acquire a %RSD < 2.

Extension of the method to Moxicip-KT

Moxicip KT ocular Drops (MOX + KET each of 0.5 % w/v) were shifted to a 10 ml graduated flask, dissolved in 0.1 M Urea, and an aliquot was diluted further to a sample solution with 10 μg/ml each of KET and MOX. For estimating the drug content, the sample spectrum was divided by the spectrum of each standard drug. The drug content was assessed using the amplitude values and the respective KET and MOX regression equations[25-26].

RESULTS AND DISCUSSION

The divisor concentrations were crucial for obtaining a good signal in the graph of the RMC process; hence, 10 μg/ml of KET and 6 μg/ml of MOX were the divisors for MOX and KET, respectively. 1.0 nm was held constant as Δλ and found to have little effect on the measurements. Each UV spectrum of MOX was divided by a zero-order spectrum of KET-10 μg/ml; all KET spectra were divided by the MOX-6 μg/ml spectrum. The spectral division was facilitated by the UV probe software, which is associated with the UV instrument software. The ratio spectral data were then subjected to mean centering using a written software program with Python and Matplotlib, as this is not available with the built-in UV software program[25,26]. The concentration (μg/ml) of MOX and KET, along with their associated amplitudes, in the RMC method was shown to be linearly related for both KET and MOX across a range of 2–10 μg/ml for each. The good correlation coefficient values (R^2=0.9993 for MOX and 0.9996 for KET) obtained for MOX and KET shows the smart linear relationship between concentrations and the corresponding amplitudes in the RMC spectra of MOX and KET. The RMC spectra for KET and MOX, respectively, are shown in Figures. 2 and 3. Table 1 provides the RMC characteristics in full.

Table 1: RMC characteristics for MOX and KET

Drug	λ(nm)	Linearity span (μg/mL)	Estimating equation, R^2
MOX	284	2 -10	y= 0.611x+0.106,0.9993
KET	314	2 -10	Y= 0. 167x+0.24,0.9996

Table 2: Recovery data

Spiked level (%)	Drug	Actual conc conc. (μg/mL)	Recovered (μg/mL) (n=3) (AM ± SD)	Recovery (%)	RSD* (%)
80	MOX	7.2	7.25± 0.11	100.69	1.51
	KET	7.2	7.26± 0.12	100.83	1.65
100	MOX	8.0	8.12 ± 0.09	101.50	1.10
	KET	8.0	8.18± 0.10	102.25	1.22
120	MOX	8.8	8.81 ± 0.11	100.11	1.25
	KET	8.8	9.06± 0.12	102.95	1.32

*Acceptance criteria = % RSD < 2

Table 3: Precision data

Drug	Conc. (μg/mL)	Precision Intra-day Experimental strength (μg/mL) (Mean ± SD) (n=3)	RSD* (%)	Precision Inter-day Experimental strength (μg/mL) (Mean ± SD) (n=3)	RSD* (%)
MOX	2	2.05 ± 0.04	0.52	2.03± 0.02	0.95
	6	6.09 ± 0.05	0.44	6.17 ± 0.05	0.27
	10	9.99 ± 0.10	0.37	10.10 ± 0.06	0..54
KET	2	2.07 ± 0.05	0.43	2.10± 0.04	0.44
	6	6.10 ± 0.06	0.54	5.90 ± 0.11	0.58
	10	10.02 ± 0.03	0.25	10.11 ±0.06	0.33

*Acceptance criteria = % RSD < 2

Tables 2 and 3 presented the computed percentage recoveries and the precision data, respectively. The percentage recoveries (%) of MOX was found to be between 100.11% and 101.5%, and for KET, it ranged between 100.83% and 102.95%. The statistical results showed good precision. The RMC approach yielded RSD values of less than 2.0% for both accuracy and precision results. The LOD and LOQ of MOX were determined to be 0.39 and 1.194 μg/mL, respectively; for KET, LOD and LOQ were found to be 0.23 and 0.718 μg/mL, respectively.

Analysis of Commercial Eye Drops (Assay)

To prove the utility of the developed method,

Table 4: Assay results of Moxicip KT

Label claim	Experimental amount (mg) Mean ± SD, RSD (%)	
MOX + KET (contains 0.5% w/v of each drug in 5 mL)	MOX	KET
	24.95 ± 0.15: 0.52%	25.38 ± 0.33; 0.78%
% Assay	99.8%	101.5%

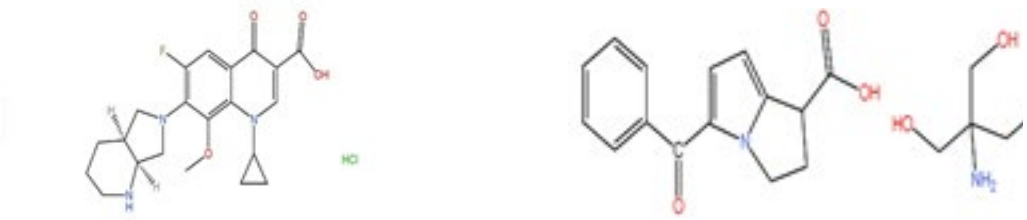

Fig. 1: Drug Structures of A. Moxifloxacin Hydrochloride & B. Ketorolac tromethamine

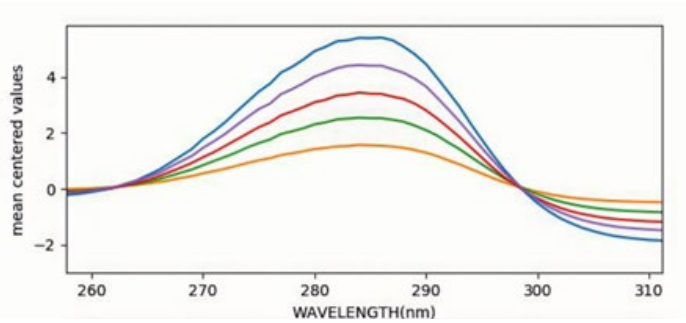

Fig. 2: Ratio mean centered spectra of MOX

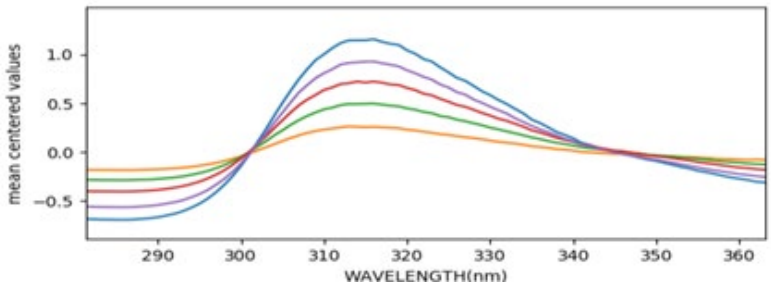

Fig. 3: Ratio mean centered spectra of KET

we analysed the marketed Moxicip KT eyedrops. **Table 4** presents the comparison between the acquired values for MOX and KET in Moxicip KT eye drops and the respective labelled amounts. The correctness of the suggested approach was demonstrated by the formulation's assay results, which showed a % RSD of less than 2.

CONCLUSION

A chemometrics-based UV spectroscopic strategy has been developed for the concurrent analysis of MOX and KET in ocular drops. For the ratio mean centring, we employed a software program based on Python and Matplotlib. It is a simple process that does not require any skilled operation as the operation of UV instrument is very handy. Since we have used 0.1M Urea as the solvent throughout the process, the method falls in the category of green analytical method.

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Conflict of Interest

The researchers disclosed the absence of any conflicts of interest.

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