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Derivative and derivative ratio spectrophotometric methods for the simultaneous determination of moxifloxacin hydrochloride with ketorolac tromethamine and ciprofloxacin hydrochloride with dexamethasone sodium phosphate in bulk and drop

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ABSTRACT

Two simple and rapid spectrophotometric methods were developed for the determination of two binary mixtures: moxifloxacin hydrochloride (MFH) with ketorolac tromethamine (KTM) and ciprofloxacin hydrochloride (CFH) with dexamethasone sodium phosphate (DSP). The first method, zero-crossing derivative spectrophotometry, depends on measuring the second derivative peak and trough values at 304 nm and 250.6 nm for MFH and KTM, respectively. The second method is a derivative ratio spectrophotometry. It depends on measuring the trough amplitude of the second derivative of the ratio spectra at 287.5 nm by dividing the spectra of CFH by the spectrum of 15.0 µg mL^{-1} DSP. Also, DSP was determined by measuring the peak amplitude of the third derivative of the ratio spectra at 254.5 nm using 10.0 µg mL^{-1} CFH as the divisor. The proposed methods were validated in compliance with the ICH guidelines and were successfully applied for determination of moxifloxacin, ciprofloxacin, ketorolac and dexamethasone in their laboratory prepared mixtures and pharmaceutical formulations.

Keywords: Moxifloxacin; ciprofloxacin; Ketorolac; Dexamethasone; Derivative spectrophotometry; Derivative ratio spectrophotometry.

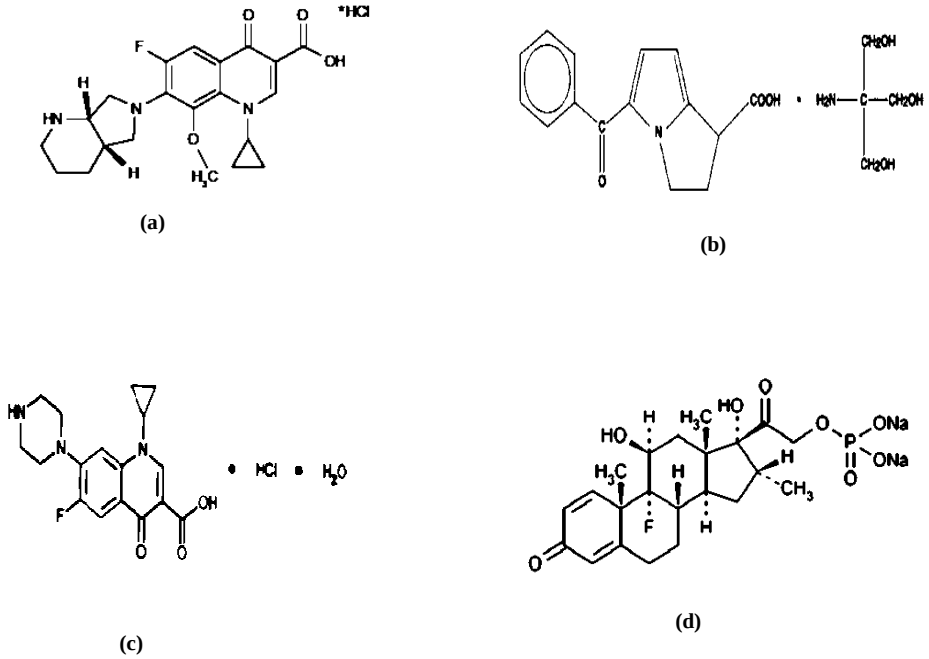
INTRODUCTION

Bacterial infection of the eye occurs in several ways, ranging from mild, self-limiting conditions to those that could be extremely serious and visually threatening. In some instances, management of patients with bacterial eye infection may involve nothing more than supportive and palliative therapy; however, in other instances, it may require aggressive intervention with antimicrobial and anti-inflammatory agents. A wide variety of antibiotics and combination antibiotic-steroid therapeutic topical formulations are available to combat bacterial infections. Antibiotic-steroid combination drugs offer a protection against further infection as well as a substantial dose of anti-inflammatory activity to suppress the body's immune response. However, quantitative determination of each drug in the combination is challenging to provide a formulation with optimum therapeutic effect. The use of these combinations overcomes the microbial resistance against common classes of antibiotics which is increasingly important global problem [1] as it is a significant phenomenon in terms of its clinical and economic impact. Patients who were infected with resistant organisms had longer hospitalizations than those infected with susceptible bacteria. In addition, increased costs were associated with infection caused by resistant species and increased mortality, despite the fact that patients received appropriate antimicrobial therapy [2].

The spectrophotometric methods have the advantages of being the most simple, fast and applicable in all laboratories, as most of the active compounds show absorbance in the UV region. But, usually compounds are present in the form of mixtures through which they exhibit strongly overlapped spectra that impede their simultaneous determination. Different manipulating techniques for absorption such as using different order derivatives [3-5], derivatives of the ratio spectrum [3,4,6,7], ratio subtraction [8,9], dual wavelength [10,11] and chemometric assisted techniques [5,6,7] gave a solution for this problem.

Moxifloxacin hydrochloride (MFH), [1-cyclopropyl-7-(S, S)-2, 8-diazabicyclo (4.3.0)-non-8-yl-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid hydrochloride] (scheme.1a), is a synthetic fourth-generation broad-spectrum fluoroquinolone antibiotic [12]. It acts by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV, which are involved in DNA replication and metabolism [13]. Ketorolac tromethamine (KTM){(rac)-5-benzoyl-1, 2-3H-pyrrolo [1,2a] pyrrole-1-carboxylic acid} (scheme.1b), a nonsteroidal anti-inflammatory drug, is indicated for short-term management of moderate to severe pain and shows a high incidence of side effects like gastric bleeding [13]. The primary mechanism of action responsible for ketorolac's anti-inflammatory, antipyretic and analgesic effects is the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX). The combination of ketorolac with moxifloxacin is extensively used for the treatment of postoperative inflammation and infection following cataract surgery [13].

Ciprofloxacin hydrochloride (CFH) [1-cyclopropyl- 6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride monohydrate] (scheme.1c) is a synthetic antibiotic of the fluoroquinolone drug class. It is a second generation fluoroquinolone antibacterial [14]. Ciprofloxacin hydrochloride kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein [15]. Dexamethasone sodium phosphate (DSP) (scheme.1d) [9- fluoro-11b, 17, 21-trihydroxy-16α-methylpregna-1, 4- diene-3, 20-dione 21-(dihydrogen phosphate) disodium salt] is a highly selective glucocorticoid which is widely used in ocular inflammatory diseases [16]. Dexamethasone in combination with ciprofloxacin hydrochloride is used in several anti-infective eye preparations to treat acute and sub acute conjunctivitis, keratitis and corneal ulcers caused by susceptible strains of the following aerobic gram positive and negative bacteria such as S. aureus, S. epidermidis, S. pneumonia and haemophilus influenza[16].



(a) **(b)** **(c)** **(d)**

Scheme (1): Chemical structure of (a) moxifloxacin HCl, (b) ketorolac tromethamine, (c) ciprofloxacin HCl, (d) dexamethasone sodium phosphate

Several analytical methods have been developed for the determination of moxifloxacin including HPLC [17-27] and UV spectrophotometery [28, 29]. Ketorolac was determined using HPTLC [30], HPLC [31-35] methods. However, a Few analytical methods were reported for the simultaneous determination of moxifloxacin and ketorolac in a mixture, namely, rapid liquid chromatography–electrospray ionization mass spectrometry (LC-MS) [13], HPLC using a diode array detector [35], RP-HPLC [36] and HPTLC [37].

Also, several analytical methods were suggested for the determination of ciprofloxacin including spectrophotometry [38, 39], HPTLC [40, 41], HPLC [42-45] and electrophoresis [46, 47]. Dexamethasone was determined using HPLC [48-50], TLC [51, 52] and electrophoresis [53]. Meanwhile, a few HPLC [16, 54-55] and HPTLC [15] methods, were reported for the simultaneous determination of ciprofloxacin and dexamethasone.

To the best of our knowledge there have not been spectrophotometric methods reported for the simultaneous determination of moxifloxacin in combination with ketorolac or for ciprofloxacin in combination with dexamethasone. Thus, we report, for the first time, on the development of derivative and derivative ratio spectrophotometeric methods for the simultaneous determination of moxifloxacin in combination with ketorolac, and ciprofloxacin in combination with dexamethasone. These methods provide simple, accurate and inexpensive mean for the analysis of these two binary mixtures without the need of sophisticated instruments, expensive solvents or large number of samples. The proposed methods are designed to be suitable for the quality assessment of these compounds in pharmaceutical products.

EXPERIMENTAL SECTION

2.1. Apparatus

Shimadzu Ultraviolet/Visible Recording Spectrophotometer 1601 (Japan), connected to an IBM compatible computer and supported with UV Probe software version 2.21.

The absorbance spectra of test and reference solutions were recorded in 1-cm quartz cells over the range 200–400 nm.

2.2. Materials and Reagents

All chemicals and reagent are of analytical or HPLC grade.

Moxifloxacin hydrochloride working standard was kindly supplied from EVA Pharma pharmaceutical company (Cairo, Egypt). Ketorolac tromethamine working standard was kindly supplied from Ameriya pharmaceutical company (Alexanderia, Egypt). Ciprofloxacin hydrochloride and Dexamethasone sodium phosphate working standard were kindly supplied from Grand Pharma pharmaceutical company (10th of Ramadan, Egypt). Megacom eye drops (Promed Group pharmaceuticals, India) containing 5.0 mg mL^{-1} of each of moxifloxacin and ketorolac and Peopo-otic ear drops (Grand Pharma for Glen mark pharmaceuticals, Egypt) containing 3.0 mg mL^{-1} ciprofloxacin and 1.0 mg mL^{-1} dexamethasone were obtained from a local pharmacy. Methanol was from Scharlau, Spain. Distilled water was produced in-house (Aquatron water still, A4000D, UK).

2.3. Solutions

2.3.1. Stock standard solutions

2.3.1.1. For derivative spectrophotometry

Stock standard solutions of MFH (200 µg mL^{-1}) and KTM (200 µg mL^{-1}) were prepared in 0.1 N NaOH. The linearity and assay solutions were prepared by appropriate dilution using 0.1 N NaOH as a diluent.

2.3.1.2. For the derivative of the ratio spectrophotometry

Stock standard solutions of CFH (200 µg mL^{-1}) and DSP (200 µg mL^{-1}) were prepared in methanol. The linearity and assay solutions were prepared by appropriate dilution with methanol.

2.3.2. Laboratory-prepared mixtures

2.3.2.1. For derivative spectrophotometry

Solutions containing MFH and KTM with the concentration ratio of (1:1) were prepared by transferring aliquots from their stock solutions into a series of 10-mL volumetric flasks and the volume of each was completed to the mark with 0.1 N NaOH.

2.3.2.2. For the derivative of the ratio spectrophotometery

Solutions containing different concentrations ratios of (3:1) and (2:1) of CFH and DSP, respectively, were prepared by transferring aliquots from their stock solutions into a series of 10-mL volumetric flasks the volume was completed to mark with methanol.

2.3.3. Sample preparation

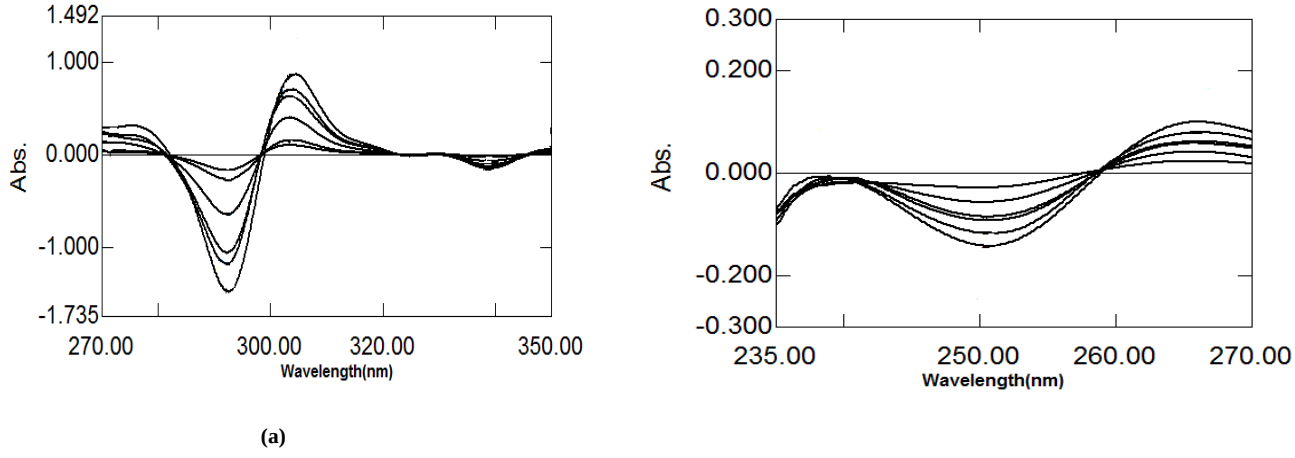
2.3.3.1. For derivative spectrophotometry

Sample solutions of MFH and KTM were prepared by transferring 1 mL of the drops to 25-mL volumetric flask. Volume was made up to the mark with 0.1N NaOH to give a concentration of 200 µg mL^{-1} of each of MFH and

KTM. Further dilution was done into 10-mL volumetric flask using 0.1 N NaOH for the quantitative determination of MFH and KTM.

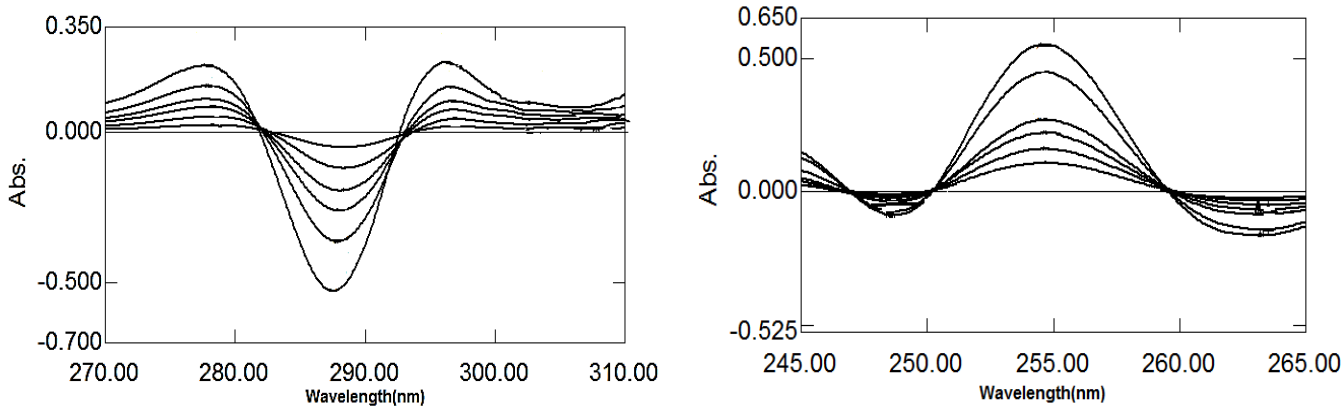
2.3.3.2. For the derivative of the ratio spectrophotometery
Sample solutions of CFH and DSP were prepared by transferring 5.0 mL of the drops into a 25-mL volumetric flask. Volume was made up to the mark with methanol to give a concentration of 600 µg mL^{-1} and 200 µg mL^{-1} for CFH and DSP, respectively. Further dilution was done into 10-mL volumetric flask using methanol for the quantitative determination of CFH and DSP.

2.4. Procedures
2.4.1. Construction of the calibration curves
2.4.1.1. For derivative spectrophotometry
Aliquots equivalent to 1.0-10.0 µg mL^{-1} of MFH and 3.0-15.0 µg mL^{-1} of KTM were accurately transferred from their stock solutions into two series of 10-mL volumetric flasks and the volumes were completed to the mark with 0.1 N NaOH. The second derivative spectra were recorded for MFH with peak amplitude measurement at 304 nm using Δλ=8 and a scaling factor =100 (Fig. 1 a). The second derivative spectra were recorded for KTM with trough amplitude measurement at 250.6 nm using Δλ=8 and a scaling factor=100 (Fig. 1b). Two calibration curves were constructed by plotting the amplitudes against the corresponding concentrations of each drug in µg mL^{-1}.



(a) **(b)**

Figure (1): (a) Second derivative spectra of moxifloxacin (1.0-10.0µg mL^{-1}) using Δλ= 8 and Scaling factor=100 (b) Second derivative spectra of ketorolac (3.0-15.0 µg mL^{-1}) using Δλ=8 and scaling factor= 100



(a) **(b)**

Figure (2): (a) Second derivative ratio spectra of ciprofloxacin (3.0-17.0 µg mL^{-1}) using dexamethasone (15.0 µg mL^{-1}) as a divisor. (b) Third derivative ratio spectra of dexamethasone (3.0-15.0 µg mL^{-1}) using ciprofloxacin (10.0 µg mL^{-1}) as a divisor

2.4.1.2. For the derivative of the ratio spectrophotometery

According to the theory of the ratio spectra derivative method, the stored UV absorption spectra of different concentrations of standard solutions of CFH (3.0-17.0 µg mL^{-1}) were divided wavelength-by wavelength by a standard spectrum of DSP (15.0 µg mL^{-1}). The second derivative was calculated for the obtained spectra with Δλ= 8 and scaling factor =1. The trough amplitudes at 287.5 nm were measured (Fig. 2a). For the determination of DSP, the stored UV absorption spectra of different concentrations of standard solutions of DSP (3.0-15.0 µg mL^{-1}) were divided wavelength-by-wavelength by a standard spectrum of CFH (10.0 µg mL^{-1}). The third derivative was calculated for the obtained spectra with Δλ=8 and a scaling factor=100. The peak amplitudes at 254.5 nm were measured (Fig. 2b).

2.4.2. Assay of laboratory-prepared mixtures

2.4.2.1. For derivative spectrophotometry

The absorption spectra of the laboratory-prepared mixtures were recorded and processed as mentioned in section 2.4.1.1 using concentration ratios equivalent to (1:1) of MFH and KTM. The concentration of each drug was calculated using the regression equation.

2.4.2.2. For the derivative of the ratio spectrophotometery

The absorption spectra of the laboratory-prepared mixtures were recorded and processed as mentioned in section 2.4.1.2 using concentrations ratios equivalent to (3:1) and (2:1) of CFH and DSP, respectively. The concentration of each drug was calculated using the regression equations.

2.4.3. Assay of Megacom eye drop

The assay concentrations were prepared by transferring 0.2 mL, 0.3 mL, 0.4 mL of the sample solution prepared in Section 2.3.3.1 to 10-mL volumetric flasks and the volume was completed to mark with 0.1 N NaOH to get concentrations equivalent to 4.0, 6.0 & 8.0 µg mL^{-1} of each of MFH and KTM. The absorption spectra of the prepared solutions were recorded, processed as mentioned in section 2.4.1.1, the amplitudes were measured and the concentrations of MFH and KTM were calculated using the regression equations.

2.4.4. Assay of Peopo-otic ear drop

The assay concentrations were prepared by transferring 0.15 mL and 0.2 mL of the sample solution prepared in Section 2.3.3.2 to 10-mL volumetric flasks and the volume was completed to the mark with methanol to give concentration ratios of (9:3) and (12:4) of CFH and DSP, respectively. The absorption spectra of the solutions were recorded, processed as mentioned in section 2.4.1.2. The amplitudes were measured and the concentrations of CFH and DSP were calculated using the regression equations.

RESULTS AND DISCUSSION

3.1. Method development

3.1.1. For derivative Spectrophotometry

The zero order absorption spectra of MFH and KTM show severe overlapping that prevents the use of direct spectrophotometry for their analysis without preliminary separation, Fig. 3. Thus, the derivative spectrophotometry was applied to solve the problem of the overlapped absorption spectra of the cited drugs. For the determination of MFH and KTM, the second derivative spectra were recorded using Δλ=8 nm and a scaling factor =100. The peak amplitudes of the obtained second derivative spectra were measured at 304 nm for MFH where KTM showed zero crossing. The trough amplitudes of the obtained second derivative spectra were measured at 250.6 nm for KTM where MFH displayed zero value, Fig. 4

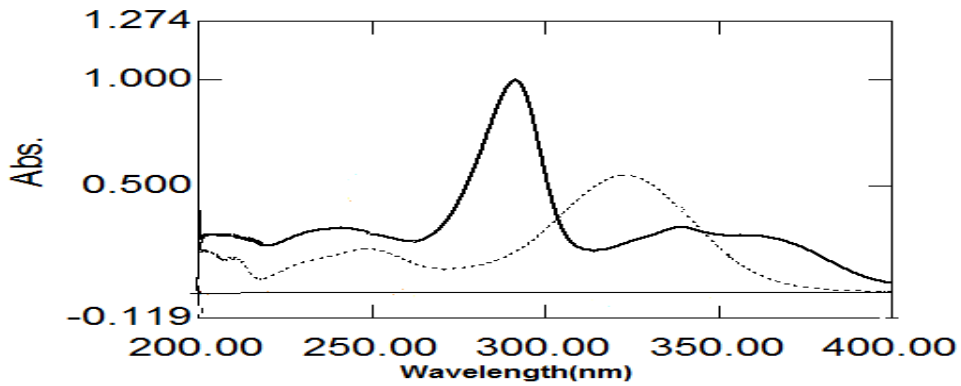


Figure (3): Zero order spectra of moxifloxacin (10.0 µg mL^{-1}) (solid line) and ketorolac (10.0 µg mL^{-1}) (dashed line)

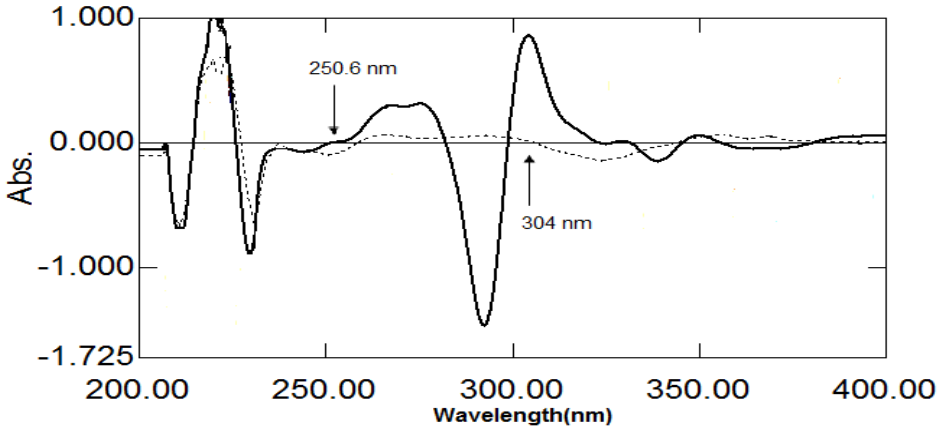


Figure (4): (a) Second derivative spectra of moxifloxacin (10.0 µg mL^{-1}) (solid line) and ketorolac (10.0 µg mL^{-1}) (dashed line) using Δλ = 8 and Scaling factor=100

3.1.2. For the derivative of the ratio spectrophotometery

The advantages of the derivative ratio spectra method over the zero-crossing derivative method, is the possibility of performing measurements in correspondence of peaks, hence, a potentially greater sensitivity and accuracy. Also in the derivative ratio method, easy measurement on the separate peaks can be carried out and there is no need to work only at zero-crossing point as in case of derivative methods [56]. Upon analysis of the mixture of CFH and DSP, overlapped zero order spectra of the two drugs (Fig.5) potentiate the need for derivatisation. Different orders of derivative spectrophotometry revealed the possibility of CFH determination due to the presence of zero crossing point of DSP, whereas, it was impossible to measure DSP in the presence of CFH throughout all the derivative orders. Thus, derivative of the ratio spectrophotometry was used instead. Unfortunately, by applying the analytical method to the pharmaceutical formulation, the vast difference in the ratio between the two drugs (CFH: DSP) (3:1) rose up as a major problem. The main parameters that affect the shape of the derivative ratio spectra are the concentration of the standard solution used as a divisor and the wavelength intervals over which the derivative is obtained (Δλ). These parameters need to be optimized to give a well resolved large peak with good selectivity and higher sensitivity in the determination [57]. The obtained ratio spectra were differentiated with respect to wavelength to afford the second and third derivative ratio spectra. Good measurements were obtained at the trough 287.5 nm and at the peak 254.5 nm amplitudes for CFH and DSP, respectively (Fig. 6 a, b). Effect of the wavelength intervals revealed that Δλ=8 was the most suitable interval for measurement of both drugs. Increasing that interval led to a less sensitive peak.

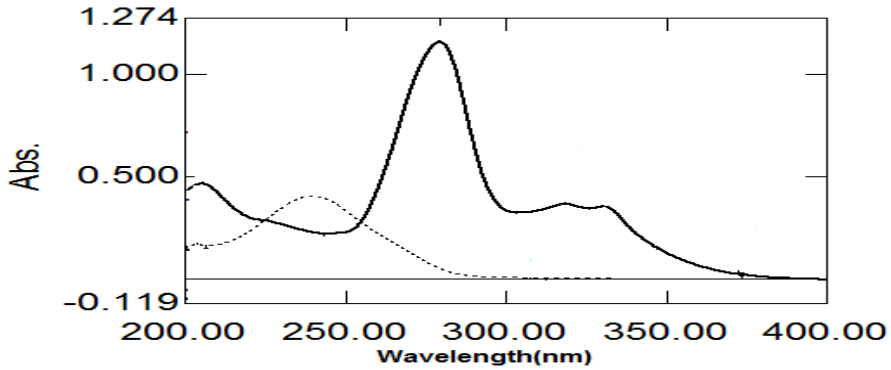
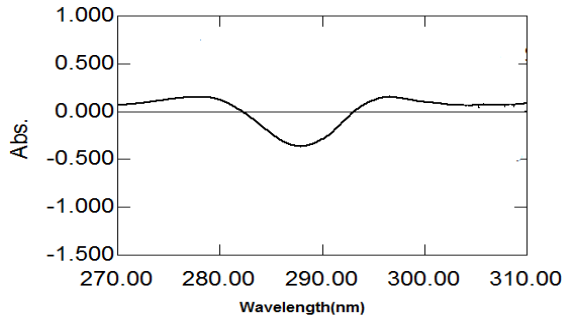
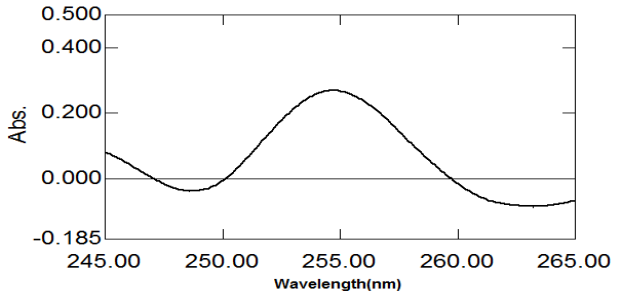


Figure (5): Zero order spectra of ciprofloxacin (10.0 µg mL^{-1}) (solid line) and dexamethasone (10.0 µg mL^{-1}) (dashed line)



(a)



(b)

Figure (6): (a) Second derivative ratio spectra of ciprofloxacin (10.0 µg mL^{-1}) using dexamethasone (15.0 µg mL^{-1}) as divisor. (b) Third derivative ratio spectra of dexamethasone (15.0 µg mL^{-1}) using ciprofloxacin (10.0 µg mL^{-1}) as divisor

3.2. Method Validation

The proposed methods were validated following the ICH guidelines [58].

3.2.1. Range and linearity

The linearity of the derivative spectrophotometric and derivative of the ratio spectrophotometric methods was evaluated by analyzing a series of different concentrations of each drug. In this study, six concentrations for MFH and KTM, ranging from 1.0-10.0 µg mL^{-1} and 3.0-15.0 µg mL^{-1}, respectively, were chosen for derivative spectrophotometric method. Six concentrations for CFH and DSP, ranging from 3.0-17.0 µg mL^{-1} and 3.0-15.0 µg mL^{-1}, respectively, were chosen for derivative of the ratio spectrophotometric method good linearity of the calibration curve was verified by the high correlation coefficient. The analytical data of the calibration curve including standard deviations for the slope and intercept (S_b, S_a) are summarized in Tables 1&2.

Table 1. Beer's law data and statistical analysis for the calibration graphs using the derivative spectrophotometric proposed method:

Item	MFH	KTM
Derivative	**^{2}D**	**^{2}D**
Solvent used	0.1N NaOH	0.1N NaOH
λ max of measurements	304 nm	250.6 nm
Concentration range	1.0-10.0 µg mL^{-1}	3.0-15.0 µg mL^{-1}
Regression equation	0.0856 C+0.0592	0.0084 C+0.0069
Regression coefficient (r^2)	0.9990	0.9994
S_b	1.32x10^{-3}	1.01x10^{-4}
S_a	8.03x10^{-3}	9.91x10^{-4}
LOD	0.12	0.93
LOQ	0.35	2.82
Confidence limit of the slope	0.0856±3.67x10^{-3}	0.0084±2.8x10^{-4}
Confidence limit of the intercept	0.0592±2.2 x10^{-2}	0.0069±2.75x10^{-3}
Standard error of the estimation	1.03x10^{-2}	9.68x10^{-4}

Table 2. Beer's law data and statistical analysis for the calibration graphs using the derivative ratio spectrophotometric proposed method

Item	CFH	DSP
Derivative ratio	**^{2}DR**	**^{3}DR**
Solvent used	Methanol	Methanol
λmax of measurements	287.5 nm	254.5 nm
Concentration range	3.0-17.0 µg mL^{-1}	3.0-15.0 µg mL^{-1}
Regression equation	0.0329 C+0.0264	0.0214 C-0.0093
Regression coefficient (r^2)	0.9996	0.9996
S_b	3.17x10^{-3}	2.1x10^{-4}
S_a	3.4x10^{-3}	2.06x10^{-3}
LOD	0.97	0.75
LOQ	2.94	2.27
Confidence limit of the slope	0.0329±8.81 x10^{-3}	0.0214±5.84x10^{-4}
Confidence limit of the intercept	0.0264±9.45 x10^{-3}	-0.0093±5.73x10^{-3}
Standard error of the estimation	3.95x10^{-3}	2.01x10^{-3}

Table 3. Determination of MFH, KTM using the derivative spectrophotometry and, CFH and DSP using the derivative ratio in bulk using the proposed methods

Drug	Conc. Taken µg mL^{-1}	Conc. Found µg mL^{-1}	Recovery %	Average recovery (mean ± SD)
MFH	4.00	4.07	101.75	101.38±0.35
	5.00	5.06	101.20	
	6.00	6.06	101.00	
	7.00	7.07	101.00	
	8.00	8.14	101.75	
	9.00	9.14	101.56	
KTM	4.00	4.01	100.25	100.79±0.43
	5.00	5.05	101.00	
	6.00	6.06	101.00	
	7.00	7.02	100.29	
	8.00	8.07	100.88	
	9.00	9.12	101.33	
CFH	12.00	12.12	101.00	100.66±0.433
	15.00	15.09	100.60	
	14.00	14.07	100.50	
	16.00	16.21	101.31	
	10.00	10.01	100.10	
	12.00	12.05	100.42	
DSP	4.00	4.03	100.75	101.26±0.43
	5.00	5.05	101.00	
	7.00	7.10	101.43	
	8.00	8.13	101.63	
	5.00	5.03	100.60	
	6.00	6.03	100.50	

Table 4. Application of standard addition technique for the determination of MFH, KTM in pharmaceutical formulation using derivative spectrophotometry and CFH, DSP using derivative ratio spectrophotometry

Drug	Found % of drug in drops[a] (mean ± SD)	Conc. Added µg mL^{-1}	Conc. Found[b] µg mL^{-1}	Recovery %	Average Recovery (mean ± SD)
MFH	98.95±0.64	2.00	1.99	99.29	99.25±0.35
		4.00	3.97	99.29	
		5.00	4.93	98.59	
		2.00	1.99	99.29	
		3.00	2.99	99.69	
		1.00	0.99	99.29	
KTM	98.76±0.50	4.00	4.05	101.19	101.35±0.29
		5.00	5.06	101.19	
		4.00	4.05	101.19	
		5.00	5.09	101.90	
		4.00	4.05	101.19	
		5.00	5.07	101.43	
CFH	101.81±0.0698	3.00	3.02	100.70	100.61±0.08
		4.00	4.02	100.61	
		5.00	5.03	100.55	
		6.00	6.03	100.51	
		4.00	4.02	100.61	
		3.00	3.02	100.71	
DSP	101.41±0.46	3.00	2.99	99.69	100.74±0.69
		4.00	4.02	100.47	
		5.00	5.05	100.93	
		6.00	6.03	100.47	
		4.00	4.02	100.47	
		3.00	2.99	99.69	

[a]*Average of six determinations*
[b]*Average of three determinations*

3.2.2. Accuracy and precision

The accuracy and precision of the proposed methods were tested by the determination of MFH, KTM, CFH and DSP at different concentration levels within the linear range of each compound. The standard addition method was applied for the determination of MFH, KTM, CFH and DSP in Megacom eye drops and Peopo-otic ear drops. The low SD (< 1) of six determinations indicated the high accuracy and precision of the proposed method. Collective results are shown in tables 3 & 4. The inter- and intra-day determination of MFH, KTM, CFH and DSP over 3 consecutive days by the same analyst using the same instrument is shown in table 5. The low RSD (< 2%) reflects the ruggedness of the methods.

Table 5. Intra- and inter-day validation for the determination of MFH, KTM, CFH and DSP using the proposed methods

Drug	Concentration µg mL^{-1}	Intra-day % RSD[a]	Inter-day % RSD[b]
	4.00		
MFH	5.00	0.54-1.36	1.61-1.76
	6.00		
	6.00		
KTM	8.00	1.00-1.65	1.65-1.89
	10.00		
	6.00		
CFH	8.00	1.18-1.85	0.397-1.53
	10.00		
	5.00		
DSP	7.00	1.47-1.69	0.83-1.93
	9.00		

[a]*The intra-day (n = 3), average of three concentrations repeated three times within the day.*
[b]*The inter-day (n = 3), average of three concentrations repeated at three consecutive days.*

3.2.5. Limit of detection and limit of quantitation

The LOD and LOQ of each method were calculated as 3.3 S/M and 10 S/M, respectively, where S is the standard deviation of the absorbance and M is the slope of the calibration curve. The data were presented in Tables 1&2.

CONCLUSION

The proposed derivative spectrophotometric and derivative of the ratio spectrophotometric methods could be successfully applied for the determination of MFH along with KTM and for CFH along with DSP without any interference and with good accuracy and precision, either in laboratory prepared mixture samples or in pharmaceutical dosage forms. All the proposed procedures are rapid, precise. The developed methods do not need sophisticated instruments, and so it can be used as alternative methods to chromatographic methods.

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