Original Article

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Simultaneous determination of ciprofloxacin hydrochloride and dexamethasone in ophthalmic solution by high performance liquid chromatography and derivative spectrophotometry

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Abstract

High performance liquid chromatography (HPLC) and derivative spectrophotometric method were developed for simultaneous determination of ciprofloxacin hydrochloride and dexamethasone in ophthalmic solution. HPLC separation was achieved in reversed-phase mode on a C18 (250 mm x 4.6 mm, 5 μm) column and using an isocratic elution of sodium octane sulfonate buffer (pH 3.8) and acetonitrile (65:35, v/v) with a flow rate of 1 mL/min. The investigated compounds were detected by a UV detector at a wavelength of 240 nm. The LC method is simple, rapid (less than 12 min.) and selective. In the derivative spectrophotometry, the first derivative ratio was obtained by using the derivation interval (Δλ) of 8 nm. The amplitude values were measured at wavelengths of 325 nm and 254 nm for the determination of ciprofloxacin hydrochloride and dexamethasone, respectively. Both methods were fully validated and applied for assay of both drugs in ophthalmic formulations. Statistical analysis was done by student t-test, which showed no significant differences (at 95% confidence interval) between the two methods.

Keyword: Ciprofloxacin hydrochloride/Dexamethasone/Derivative spectrophotometry/High-performance liquid chromatography/Ophthalmic solution

1. INTRODUCTION

Ciprofloxacin hydrochloride and dexamethasone ophthalmic solution is a new combination formula that is not available in the United States Pharmacopeia (USP). The combination of these drugs has been used to treat bacterial infections by antibiotic effect of ciprofloxacin hydrochloride and the inflammation reduction by dexamethasone[1].

Ciprofloxacin hydrochloride (Figure 1), quinolone derivative, is an antibiotic for treatment of urinary tract infection, gastrointestinal, respiratory and other infectious diseases. Dexamethasone (Figure 1) is a corticosteroid that acts as an anti-inflammation and immune-suppressant[2-3]. Both drugs are highly effective and show low rates of drug resistance. USP 37 recommends two HPLC systems for the assay of ciprofloxacin hydrochloride and dexamethasone in otic suspension[4]. However, there is no official method for simultaneous assay of both drugs in any pharmacopoeia. The current work proposes accurate, precise and reliable methods for simultaneous determination of ciprofloxacin hydrochloride and dexamethasone using reversed-phase HPLC and derivative UV spectrophotometric methods.

Derivative UV spectrophotometric method is suitable for assay two or more components with overlapping spectra. This method has been widely used as a tool for quantitative analysis, characterization and quality control of pharmaceuticals[5-12]. The advantages of this tool are simplicity, flexibility, convenience, cost and time saving[13]. Derivative UV spectrophotometric method is based on derivative

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spectra of a zero-order spectrum such as zero-crossing and ratio-spectra derivative techniques.

HPLC method is a method of choice for testing drug purity, tracking changes in the synthesis process, formulation of new pharmaceutical preparations, quality control and quality assurance of pharmaceutical products [14-20].

Presently, two analytical procedures, HPLC and derivative UV spectrophotometry techniques, were developed for simultaneous determination of ciprofloxacin hydrochloride and dexamethasone. Both methods were optimized, validated and applied to assay of ciprofloxacin hydrochloride and dexamethasone in ophthalmic solution.

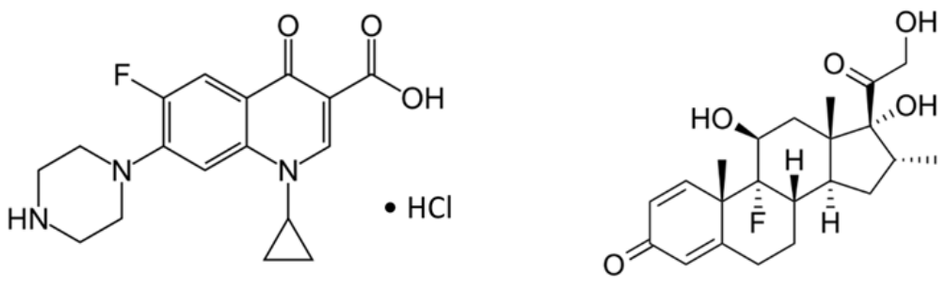

Figure 1. Chemical structure of ciprofloxacin hydrochloride (A) and dexamethasone (B)

2. MATERIALS AND METHODS

2.1 Chemicals, reagents and materials

1-Octane sulfonic acid, sodium salt (HPLC grade), acetonitrile, hydrochloric acid (AR grade) and triethylamine (synthesis grade) were from Fluka (London, UK). Acetonitrile (HPLC grade) was from RCI Labscan (Bangkok, Thailand). Ciprofloxacin hydrochloride and dexamethasone were from Zhejiang Guobang Pharmaceutical Co., Ltd. (Zhejiang, China). Ciprofloxacin ethylenediamine (USP standard, Rockville, USA). Methanol (HPLC grade) was from Burdick & Jackson Reagent Plus (Seoul, Korea). Phosphoric acid and sodium hydroxide were from Carlo Erba (Milan, Italy). High purity water was prepared by using Waters Milli-Q plus purification system.

2.2 Equipment

High performance liquid chromatography

The HPLC system consisted of LC-20 (Shimadzu, Japan) with a UV detector. The output signal was monitored and integrated using the LC Solution software. The chromatographic analysis was performed on a C18 column (250 mm x 4.6 mm i.d. dimensions and 5 µm particle size) (ACE®, USA). Detector was performed at 240 nm.

Derivative UV spectrophotometry

Derivative UV spectrophotometric analysis was performed on Spectrophotometer UV-1800 (Shimadzu, Japan). The UV spectrophotometer was set at 200 - 400 nm, slit width 1 cm, and the absorption spectra of the reference and test solutions were carried out in a 1-cm quartz cell. The UV Probe software was used to derive spectra. The method was optimized in term of derivation interval ($\Delta\lambda$) to obtain appropriate signals.

2.3 Solutions preparation for High performance liquid chromatography

2.3.1 Mobile phase preparation

The mobile phase consisted of 5 mM sodium 1-octane sulfonic acid solution (adjusted to pH 3.8 using triethylamine) and acetonitrile at the ratio of 65:35 (v/v). The mobile phase was filtered through a 0.45 µm nylon membrane and degassed prior to use.

2.3.2 Standard preparation

Standard stock solutions of ciprofloxacin hydrochloride and dexamethasone were prepared at 75 µg/mL and 25 µg/mL in 50% acetonitrile, respectively. Ciprofloxacin hydrochloride working standard solutions was prepared by diluting the stock solution with the mobile phase to obtain the concentration of 150 µg/mL. Dexamethasone working standard solutions was prepared by diluting the stock solution with acetonitrile to obtain the concentration of 50 µg/mL. The mixed standard solutions were filtered through a 0.45 µm nylon syringe filter before injection into HPLC system.

2.3.3 Sample preparation

Twenty units of commercial ciprofloxacin hydrochloride and dexamethasone ophthalmic solution were pooled. The analytical sample was prepared by diluting the pooled sample with the mobile phase to obtain the concentration of ciprofloxacin

hydrochloride at 3 µg/mL and dexamethasone at 1 µg/mL. Each sample solution was filtered through 0.45-µm nylon syringe filter before injection into HPLC system.

2.4 Solutions preparation for derivative UV spectrophotometry

2.4.1 Standard preparation

Standard stock solutions of ciprofloxacin hydrochloride and dexamethasone were prepared in 50% acetonitrile to obtain the concentration of 75 µg/mL and 25 µg/mL for ciprofloxacin hydrochloride and dexamethasone, respectively.

2.4.2 Sample preparation

Twenty units of commercial ciprofloxacin hydrochloride and dexamethasone ophthalmic solution were pooled. The analytical sample solutions were prepared by diluting the pooled sample in 50% acetonitrile to obtain the concentration of ciprofloxacin hydrochloride at 12 µg/mL and dexamethasone at 4 µg/mL.

2.5 Methods

2.5.1 High performance liquid chromatography

The HPLC experiment was performed using a C18 Column (250 mm x 4.6 µm, i.d. 5 µm) analytical column. 5 mM sodium 1-octane sulfonic acid solution (adjusted to pH 3.8 using triethylamine) and acetonitrile at the ratio of 65:35 (v/v) was delivered through the column at the flow rate of 1.0 ml/min. The sample injection volume was 20 µl. The UV-detector was set at a wavelength of 240 nm.

2.5.2 Derivative UV spectrophotometry

Derivative UV spectra for the solution of ciprofloxacin hydrochloride and dexamethasone were recorded in a 1 cm quartz cell over the range 200 - 400 nm using 50% acetonitrile in the reference cell. Each spectrum was recorded in triplicate.

2.5.3 Method validation

To validate HPLC and UV spectrometric methods, system suitability (for HPLC method), specificity, sensitivity, linearity, precision and reproducibility were assessed. System suitability was evaluated by taking data from five replicates analyses of a mix standards (ciprofloxacin hydrochloride, dexamethasone and ciprofloxacin ethylenediamine). Parameters used for monitoring system suitability were plate numbers (N), resolution (R_s), tailing factors (Tf), %RSD of peak area and retention time (tR), all calculated using the United States Pharmacopeia (USP) equations. The linearity of both methods were assessed by linear regression analysis. For UV spectrometric method, linearity was studied by analyzing six standard solutions covering the range of 5.71 – 17.13 µg/mL for ciprofloxacin hydrochloride and 1.99 – 5.98 µg/mL for dexamethasone (n = 3). For HPLC method, linearity was checked by preparing series of standard solutions of both ciprofloxacin hydrochloride and dexamethasone. The calibration curves of two drugs were established using five standard solutions covering the range 1.5 – 4.5 µg/mL and 0.5 – 1.5 µg/mL for ciprofloxacin hydrochloride and dexamethasone, respectively. Each sample was injected in triplicate. Calibration data were calculated from the area under peak measurement at 240 nm for both drugs. Standard addition method was performed in ophthalmic

solution to assess the accuracy and precision of the methods. Accuracy was showed by % recovery (*%R*) while repeatability was expressed as the relative standard deviation (%RSD) of intra- and inter-day precision. For UV spectrometric method, repeatability was determined at three points of the calibration curve (the concentration of 5.72, 11.43, 17.16 µg/mL for ciprofloxacin hydrochloride (n = 6) and concentration of 1.96, 3.92, 5.89 µg/mL for dexamethasone (n = 6)). Intra-day and inter-day variability was determined by analyzing six standard solutions of the calibration curve (n = 6) within one day and on three different days (n = 6), respectively. For HPLC method, repeatability was determined at three points of the calibration curve (the concentration of 1.28, 2.57, 3.85 µg/mL for ciprofloxacin (n = 6) and concentration of 0.49, 0.98, 1.47 µg/mL for dexamethasone (n = 6)). Each concentration was analyzed in triplicates. System sensitivity was assessed using limits of detection (LOD) and quantification (LOQ). For spectrophotometric method LOD and LOQ were calculated by equation LOD = $3.3\delta / s$ and LOQ = $10\delta / s$, respectively, where δ is the standard deviation of blank and s is slope of calibration curve. For HPLC method, ciprofloxacin ethylenediamine was used as internal standard for the purpose of quantification of co-formulated drugs. LOQ and LOD were calculated by LOQ = 10 (SD/ s) and LOD = 3.3 (SD/ s)**,** where: SD = the standard deviation of y-intercept of calibration curve and s = the slope of calibration curve**.**

3. RESULTS AND DISCUSSION

3.1 High performance liquid chromatography

For HPLC condition development, obtaining acceptable peak parameters for the analytes is essential in quantitative studies. Initially the condition from USP 37[4] for analysis of ciprofloxacin hydrochloride in ciprofloxacin hydrochloride and dexamethasone otic suspension monograph was investigated. The column was L1(C18) 150 x 3.9 mm I.D. and the mobile phase was the mixture of 0.05 M phosphate buffer pH 3.0 (with 0.4% diethylamine) and acetonitrile (89:11). The flow rate was 1.5 mL/min. The detection wavelength was 280 nm. The injection volume was 20 µL. The system was not suitable for the analysis. The tailing factor of ciprofloxacin hydrochloride was more than 2 and the signal of dexamethasone was low.Various ratios, types and pH of buffer and acetonitrile were tried to obtain the optimum separation and good peak shapes of ciprofloxacin hydrochloride, dexamethasone and ciprofloxacin ethylenediamine (internal standard). The addition of sodium 1-octane sulfonate as an ion pairing reagent to the mobile phase and vary the ratio of buffer and acetonitrile can improve resolution and peak parameters. The optimum isocratic mobile phase was 5 mM sodium 1-octane sulfonate solution (pH 3.8) and acetonitrile (65:35) with a flow rate of 1.0 mL/min. UV detection wavelength is 240 nm. The analytes were based separated (resolution = 4.30 and 17.80, calculated from ciprofloxacin ethylenediamine peak, ciprofloxacin hydrochloride peak and dexamethasone peak, respectively) in less than 12 min. Peak shapes were satisfactory with the tailing factor of 1.50. The retention times of ciprofloxacin hydrochloride and dexamethasone were about 5 and 10 min, respectively (Figure 2).

3.2 Derivative UV spectrophotometric method

Both ciprofloxacin hydrochloride and dexamethasone were completely soluble in 50% acetonitrile, whereas dexamethasone solution was turbid in the mixture of phosphoric acid buffer with acetonitrile. 50% Acetonitrile was selected as the solvent for both drugs. This solvent provided the highest solubility and the UV spectrum without interferences from sample matrix for both direct UV and first derivative measurements. The zero order spectra of standard ciprofloxacin hydrochloride and dexamethasone were overlapped (Figure 3). For solving this problem the ratio spectra derivative spectrophotometry was used. Calculation the ratio of the standard ciprofloxacin hydrochloride absorbance (5 concentrations) divided by the absorbance of fixed concentration of standard dexamethasone (wavelength by wavelength). The ratio spectra were manipulated to derive the first order ratio spectra (Figure 4). The same procedures were calculated for dexamethasone standard spectra

to obtain first order ratio spectra of dexamethasone (Figure 5). The maximum wavelength (λ_{max}) for ciprofloxacin hydrochloride was 325 nm and the minimum wavelength for dexamethasone was 254 nm. For first derivative, the derivation interval ($\Delta\lambda$) was selected at 8 nm.

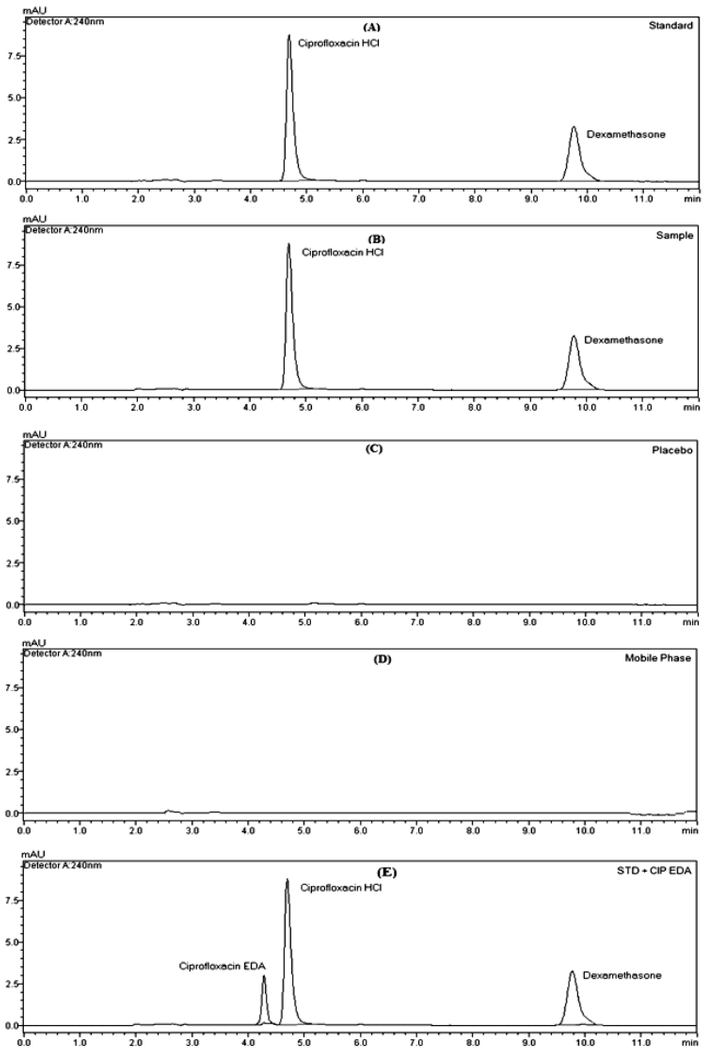

Figure 2. HPLC chromatogram of 20 µL injector of the standard solution (A), the ophthalmic solution of ciprofloxacin hydrochloride 3 µg/mL and dexamethasone 1 µg/mL (B), placebo solution (C), mobile phase (D) and the standard solution spiked with ciprofloxacin ethylenediamine (ciprofloxacin EDA) (E)

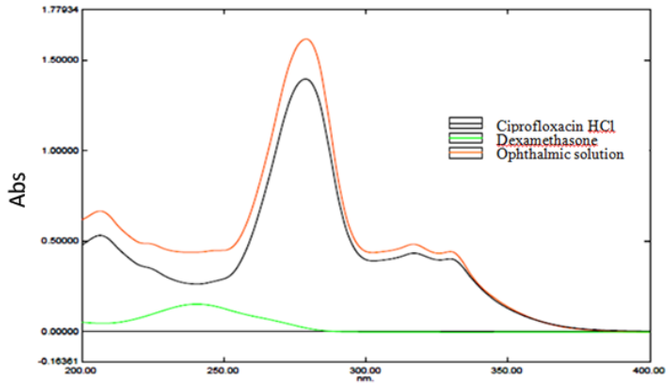

Figure 3. Zero order spectrum of ciprofloxacin hydrochloride 12 µg/ml, dexamethasone 4 µg/ml and the ophthalmic solution contain both drugs at the same concentrations

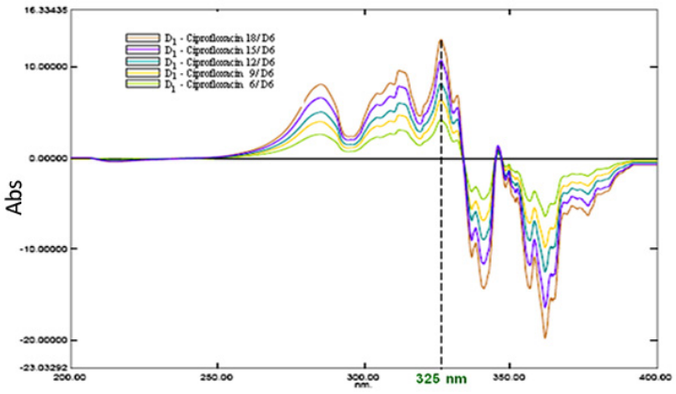

Figure 4. First derivative ratio spectra of ciprofloxacin hydrochloride (6 - 18 µg/mL), divisor is 6 µg/mL dexamethasone

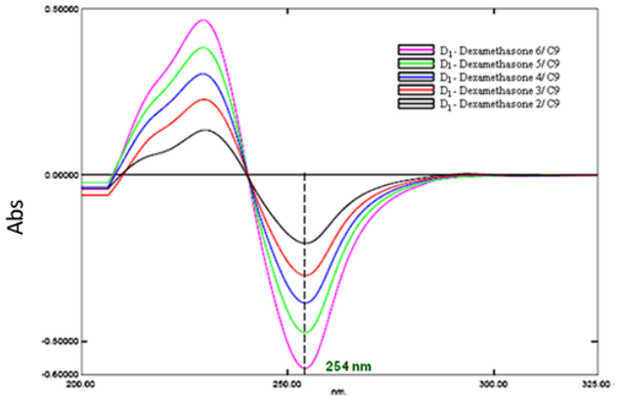

Figure 5. First derivative ratio spectra of dexamethasone (2 - 6 µg/mL), divisor is 9 µg/mL ciprofloxacin hydrochloride

3.3 Method validation

System suitability was performed to verify the chromatographic system was adequate for the analysis. The capacity factor value (K'), the efficiency of column as expressed by plate numbers (N), tailing factor (Tf) and the baseline separation expressed as resolution (R_s) were all in the acceptable range (N >2000, 0.5≤ Tf ≤ 2 and R_s > 2) . Plate numbers (N) for all analytes were greater than 7,400. Resolutions (R_s) were better than 4.30 and tailing factors were equal to 1.50 for both ciprofloxacin hydrochloride and dexamethasone. % RSD of peak area and retention time (tR) were within 0.05 and 0.05, respectively, which indicated the suitability of the system. The regression statistic were calculated from the calibration curves constructed for standard ciprofloxacin hydrochloride and dexamethasone from both methods showed satisfactory linearity with the correlation coefficient (r^2) greater than 0.999 for all. For HPLC method, the calibration curves of two drugs were established using five standard solutions covering the range 1.5 – 4.5 µg/mL and 0.5 – 1.5 µg/mL for ciprofloxacin hydrochloride and dexamethasone, respectively. For first derivative ratio spectrophotometric method, the calibration curves were established using five standard solutions covering the range 6 – 18 µg/mL and 2 – 6 µg/mL for ciprofloxacin and dexamethasone, respectively. The precision of the methods were evaluated by intra-day and inter-day repeatability. The % RSD of first derivative ratio spectrophotometric method was within 1.56% and % RSD of the HPLC method were less than 1.42% and 0.05% for peak area and the retention time, respectively. LOD and LOQ from first derivative ratio spectrophotometric method of ciprofloxacin hydrochloride were 0.18 and 0.55 µg/mL and for dexamethasone were 0.15 and 0.45 µg/mL. For HPLC method, LOD and LOQ of ciprofloxacin hydrochloride were 0.08 and 0.23 µg/mL and for dexamethasone were 0.03 and 0.08 µg/mL. Recoveries of the methods were performed in commercial ophthalmic solution using standard addition method covering the range of 50 – 150% of the calibration curve concentration. For HPLC method, % recoveries were within 101.84 – 103.18% (% RSD = 0.55 – 0.80%) and 97.89 – 100.64% (% RSD = 0.24 – 0.52%) for ciprofloxacin and dexamethasone, respectively. For first derivative ratio spectrophotometric method, % recoveries were within 100.32 – 102.65% (% RSD = 0.36 – 0.81%) and 96.52 – 97.13% (% RSD = 0.17 – 0.61%) for ciprofloxacin and dexamethasone, respectively. The selectivity of the HPLC method was confirmed (Figure 2). Mobile phase, placebo solutions, the standard solution (ciprofloxacin hydrochloride and dexamethasone) and the standard solution spiked with ciprofloxacin ethylenediamine

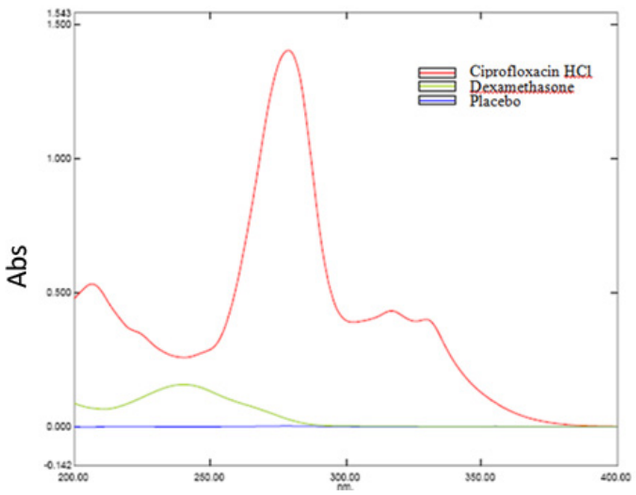

Figure 6. Zero order spectrum of ciprofloxacin hydrochloride, dexamethasone and placebo

(ciprofloxacin EDA) were injected to confirm additionally the specificity of method. Results showed no interference from the placebo. For first derivative ratio spectrophotometric method, the zero-order absorption spectra of ciprofloxacin hydrochloride, dexamethasone and placebo in 50% acetonitrile are shown in Figure 6. The spectra display over lapping in the region of 200 to 300 nm. This makes the determination of ciprofloxacin hydrochloride in presence of dexamethasone by conventional UV spectrophotometry difficult, but the determination of ciprofloxacin hydrochloride from 310 to 375 nm might be possible without the interference from dexamethasone. The other compounds in preparation (placebo) were not interfered the spectrum. The derivative ratio spectrophotometry technique was chosen for the determination of both drugs in order to overcome the interference from peak overlapping. Derivative spectra of first derivative ratio were used to determine both ciprofloxacin hydrochloride and dexamethasone simultaneously.

3.4 Application for analysis of ciprofloxacin hydrochloride and dexamethasone in ophthalmic solution

The developed derivative UV spectrophotometric method was applied for the analysis of ciprofloxacin hydrochloride and dexamethasone in ophthalmic solution comparing with the developed HPLC method. Twenty units of sample from the same batch were pooled. The eighteen samplings were used for determined the difference of the two methods. The results show no degradation products were observed during the analysis. Results from the assay of ciprofloxacin hydrochloride and dexamethasone by derivative UV spectrophotometric method and HPLC method are shown in Table 1. Typical chromatograms and spectra of ciprofloxacin hydrochloride and dexamethasone in sample are shown in Figure 2 and 3, respectively. The calculated t-test indicated that there were no significant difference between HPLC and derivative spectrophotometric method with regard to quantitative analysis, accuracy and precision. However, LOD and LOQ of HPLC technique obtained lower levels than derivative spectrophotometric method for both drugs but derivative spectrophotometry gave the wider range of concentration of analytes than HPLC (Table 2). Thus, both methods are applicable for the determination of ciprofloxacin hydrochloride and dexamethasone in commercial ophthalmic solutions.

Table 1. Comparison of ciprofloxacin hydrochloride and dexamethasone contents in ophthalmic solution (%LA) by derivative UV spectrophotometric method and HPLC method

	Ciprofloxacin		Dexamethasone	
	D_1 at 325 nm	HPLC	D_1 at 254 nm	HPLC
Mean + SD	100.77+1.58	101.08+0.39	97.14+1.17	96.75+0.37
Variation	2.4839	0.1508	1.3801	0.1404
Hypothesized Mean Difference	0		0	
t Stat	-0.8030		1.2695	
P(T<=t) two-tail	0.4319		0.2214	
t Critical two-tail	2.0930		2.1098	

hypothesis: no difference between the means
$t_{calc} < t^{table}$ (P > 0.025) then accept the hypothesis at 95% confidence level
The means of quantitative of two drugs were not difference by the derivative spectrophotometric method and the HPLC method at 95% confidence level.

Table 2. Validation data of the developed first derivative ratio spectrophotometric and HPLC methods

Validation Parameter	Ciprofloxacin		Dexamethasone	
	D_1 at 325 nm	HPLC	D_1 at 254 nm	HPLC
Slope	0.3093	23,207.478	- 0.0924	49,000.028
Intercept	– 0.0700	+ 98.496	– 0.0013	+ 114.840
Correlation coefficient (r^2)	0.9997	0.9996	1.0000	0.9998
Intra-day precision (n=6)	0.67 – 1.68	0.37 - 1.31	0.76 – 1.70	0.12 - 0.47
Inter-day precision (n=6)	1.56	1.11	1.21	0.39
LOD (µg/mL)	0.18	0.08	0.15	0.03
LOQ (µg/mL)	0.55	0.23	0.45	0.08
% Recovery (%RSD)	101.12% (<0.81)	102.34% (<0.77)	96.80% (<0.61)	99.12% (<0.54)

4. CONCLUSIONS

The validated parameters prove that the two proposed methods are accurate, precise, reproducible and selective for the ophthalmic solution formula. The two proposed methods based on the derivative UV spectrophotometric and reversed-phase HPLC are suitable for simultaneous determination of ciprofloxacin and dexamethasone in the eye drop preparation. Comparison of the two methods by *t*-test shows no significant difference in 95% confidence. Although the HPLC method is a good separation technique for quantification of the both drugs which gave runtime less than 12 minutes. The spectrophotometric method required only fundamental UV spectrophotometer to operate the wavelength scan and used the software to calculation the first derivative value. Also, it is accurate, precise and time saving (cause no spent time to separation) and therefore it can be considered alternative tools for the routine analysis or in-process control of commercial ophthalmic solution containing the both drugs.

5. ACKNOWLEDGEMENTS

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