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Comparative Study of RP–HPLC Versus TLC–Spectrodensitometric Methods Applied for Binary Mixtures of Fluoroquinolones and Corticosteroids

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Summary. Reversed phase high-performance liquid chromatography (RP-HPLC) and thin-layer chromatography (TLC)-spectrodensitometric methods have been developed and validated for the separation and quantitation of two binary mixtures: Ofloxacin (OFX) and dexamethasone (DXM) in eye preparation; ciprofloxacin hydrochloride (CIP) and hydrocortisone (HYD) in ear preparation. The linearity ranges of RP-HPLC methods were found to be (2.5–45 μg mL^{-1}) for OFX, (2.5–50 μg mL^{-1}) for DXM and (1–8 μg mL^{-1}) for both CIP and HYD. The percentage recoveries/relative standard deviation (RSD) were found to be 100.36/1.38, 100.13/1.49, 99.98/0.61 and 100.28/1.27, respectively. The linearity ranges of TLC-spectrodensitometric methods were found to be (0.5–2 μg $band^{-1}$), (0.5–3.5 μg $band^{-1}$), (0.2–1.6 μg $band^{-1}$), and (0.6–2 μg $band^{-1}$) for OFX, DXM, CIP, and HYD, respectively. The percentage recoveries/RSD were found to be 99.98/1.06, 99.93/1.18, 99.74/1.27, and 99.94/1.54, respectively. A comparative study was conducted to show the advantages of the proposed methods which showed that the TLC-spectrodensitometric methods were simpler, more sensitive, and economic, while RP-HPLC methods were more precise and robust. The methods were validated in compliance with the ICH guidelines and were successfully applied for determination of the selected drugs in their laboratory-prepared mixtures and commercial dosage forms.

Key Words: ofloxacin, dexamethasone, ciprofloxacin hydrochloride, hydrocortisone, HPLC, TLC-spectrodensitometry

Introduction

Ofloxacin (OFX) [9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido (1, 2, 3-de)-1, 4-benzoxazine-6-carboxylic acid] and ciprofloxacin hydrochloride (CIP) [1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid] are fluoroquinolone antibacterial agents which are active against a wide range of bacteria. Dexa-

methasone (DXM) [9α-Fluoro-11β, 17α, 21-trihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione] and hydrocortisone (HYD) [(11β)-11, 17, 21-trihydroxypregn-4-ene-3, 20-dione] are corticosteroids with anti-inflammatory effect [1, 2]. The chemical structures for these drugs are shown in *Fig. 1*. The combinations of OFX and DXM (mixture A) are being used as anti-infective eye preparations to treat acute and sub-acute conjunctivitis, keratitis, and corneal ulcers, while the combinations of CIP and HYD (mixture B) are being used as ear preparations to treat outer ear infections and reduce swelling.

Different methods describing the determination of the fluoroquinolones have previously been reported such as spectrophotometry [3–5], HPLC [6–8], thin-layer chromatography (TLC) [9], and capillary electrophoresis [10]. Other methods were reported for the corticosteroids, such as spectrophotometry [11, 12], HPLC [13–16], and capillary electrophoresis [17, 18].

HPLC methods have been reported for the determination of mixture A [19–21] and mixture B [22, 23]. The reported methods suffered of a lack of robustness as the conditions, the mobile phase ratio, and composition seemed rather critical, so they could not be easily applied in quality control laboratories. No TLC-spectrodensitometric methods have been reported for the analysis of these mixtures.

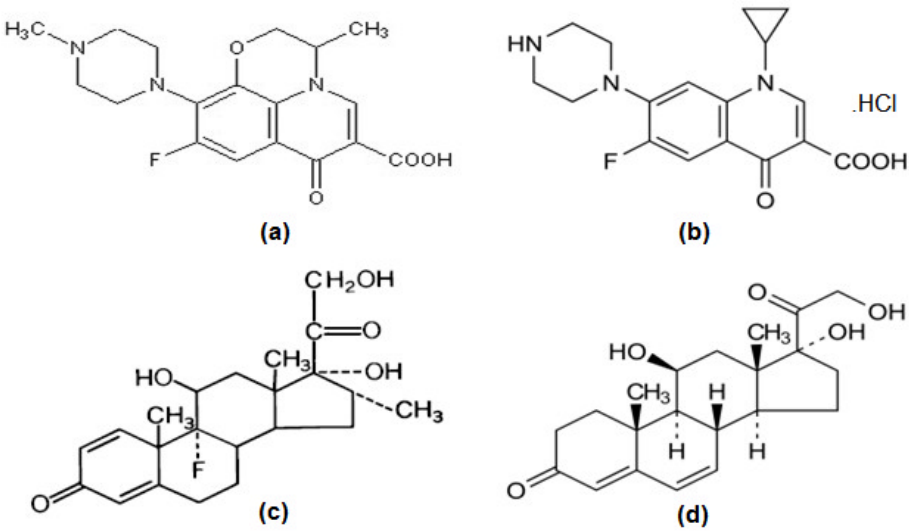

Fig. 1. The chemical structures of (a) OFX, (b) CIP, (c) DXM, and (d) HYD

The aim of this work was to develop and validate reversed phase high-performance liquid chromatography (RP-HPLC) and TLC-spectrodensitometric methods for resolving those mixtures. The methods were simple,

accurate, precise, and robust. A comparative study was conducted between the developed methods to compare between them and show their advantages over the reported ones.

Experimental

Apparatus

The HPLC system: Agilent 1200 series chromatographic system equipped with quaternary pump, microvacuum degasser, thermostatted column compartment, and variable wavelength UV-VIS detector was used. Sample injections were made through an Agilent 1200 series autosampler. Data collection and processing were performed using Agilent ChemStation software, version A.10.01. Column Agilent Zorbax SB-C_{18} (150 × 4.6 mm, 5 μm particle size i.d.) was from (Agilent Technologies, Polo Alto, CA, USA. A "Jenway 3505" pH-meter (Jenway, UK), equipped with combined glass electrode was used for pH adjustment.

The TLC-spectrodensitometry system: Camag TLC scanner 3 S/N 130319 operated with winCATS software, Linomat 5 autosampler (Switzerland), Camag microsyringe (100 μL) and TLC aluminum sheet (20 × 20 cm) precoated with silica gel 60 F_{254}. (Merck KgaA, Darmstad, Germany) were used.

Materials

Samples

OFX, CIP, and DXM reference standards were kindly supplied by Egyptian International Pharmaceutical Industries Co. (EIPICO), Cairo, Egypt, while HYD reference standard was kindly supplied by Sigma Pharmaceutical Industries Limited, Al-Monofeya, Egypt. Their purities were found to be 100.21 ± 0.95, 99.86 ± 0.85, 101.82 ± 1.09, and 100.52 ± 0.64 for OFX, CIP, DXM and HYD, respectively, by official methods [2]. Dexaflox® eye drops (3 mg of OFX, 1 mg of DXM, and 0.06 mg of Benzalkonium chloride per 1 mL, batch number: LB060) was manufactured by Jamjoompharma, Kingdom of Saudi Arabia. Ciprocort® ear drops (10 mg of HYD and 2.3 mg of CIP per 1 mL, batch number: 1601001) was manufactured by European Egyptian Pharmaceutical Industries (EEPI), Alexandria, Egypt. Both samples were purchased from the local market.

Chemicals

Sodium hydroxide scaled, ethyl acetate, 33% ammonia solution and ortho phosphoric acid (85%) were supplied by Adwic-El Nasr pharmaceutical Chemicals Co., Egypt). Methanol, chloroform, and triethylamine were supplied by Lobachemie, India. Acetonitrile (HPLC grade) was supplied by LabScan Limited, Dublin, Ireland.

Standard Solutions

Stock solutions of OFX and DXM were prepared using methanol, of concentration 1 mg mL^{-1}, while stock solutions of CIP and HYD were prepared using a solvent mixture of methanol–water (80:20 v/v), of concentrations 1 and 0.5 mg mL^{-1}, respectively. All solutions were stored in dark bottles at 4°C and were stable for 3 weeks. Their stability was tested using reported methods [8, 15].

Working Solutions

For RP-HPLC Methods

Working solutions were freshly prepared by dilution from the stock solutions with the mobile phase to obtain a concentration of (50 μg mL^{-1}) for OFX and DXM and (10 μg mL^{-1}) for CIP and HYD.

For TLC-Spectrodensitometric Methods

Working solutions were freshly prepared by dilution from the stock solutions with the same solvent to obtain a concentration of (500 μg mL^{-1}) for OFX and DXM and (200 μg mL^{-1}) for CIP and HYD.

Procedure

RP-HPLC Methods

Chromatographic conditions. RP-HPLC was carried out at ambient temperature on Zorbax SB-C_{18} column. For mixture A: the mobile phase consisted of acetonitrile: pH-controlled solution (pH 3.3) in the ratio of (45:55 v/v). The solution was made up by adding 280 μL triethylamine and 1.4 mL o-phosphoric acid to 1000 mL water, and the pH was adjusted to 3.3 using 10% sodium hydroxide solution. For mixture B: the mobile phase consisted of acetonitrile–distilled water–pH-controlled solution (pH 3) in the ratio of

(55:40:5 *v*/*v*/*v*). The pH-controlled solution was composed of 1% triethylamine in water, and the pH was adjusted to 3 using o-phosphoric acid. The mobile phases were filtered using 0.45 μm Millipore membrane filter (Billerica, MA) and delivered at a rate of 0.6 mL min^{-1}. The injection volumes were 20 μL, and the detections were done at 240 nm (for mixture A) and 256 nm (mixture B).

System suitability. Twenty microliters of the working solutions were injected and applied to the chromatographic conditions. The system suitability parameters including retention time, tailing factor, theoretical plate count (N), height of theoretical plate (HETP), and resolution were calculated according to USP guidelines [24].

Construction of calibration curves. Separate aliquots were transferred from the working solution of each drug to prepare solutions of different concentrations. The corresponding chromatographic conditions were applied for these solutions, and the chromatograms were recorded. The calibration curve of each drug was constructed by plotting the relative peak area (the peak area found to that of an external standard of the same drug) against the corresponding concentration, from which the regression equations were calculated. For mixture A, the calibration curves for OFX and DXM were constructed using the external standards (2.5 μg mL^{-1} of OFX and DXM) at 240 nm. For mixture B, the calibration curves for CIP and HYD were constructed using the external standards (1 μg mL^{-1} of CIP and HYD) at 256 nm. Calibration curves were constructed in the range of 2.5–45 μg mL^{-1} for OFX, 2.5–50 μg mL^{-1} for DXM, and 1–8 μg mL^{-1} for both CIP and HYD. The calibration curves were constructed using the average of three experiments.

TLC-spectrodensitometric methods

Chromatographic conditions. TLC aluminum sheets 20 × 10 cm precoated with 0.25 mm silica gel 60 F254 were used. The samples were applied to the TLC plate as bands (bandwidth: 6 mm, bands were spaced 1 cm apart from each other and 1 cm from the bottom edge of the plate). The applied volume per band was 10 μL using a 100-μL syringe. For mixture A: the developing system was chloroform–methanol–triethylamine (35:10:10 *v*/*v*/*v*). For mixture B: the developing system was ethyl acetate–hexane–triethylamine (50:25:25 *v*/*v*/*v*). Linear ascending development was done in a chromatographic tank previously saturated with the developing system for 1 h at room temperature to a distance of approximately 8 cm from the lower edge. The developed plates were air dried and scanned at 240 nm for mixture A and at 243 nm for mixture B. The detection was done using Camag TLC scanner 3 operated in the absorbance mode, with deuterium lamp as a

source of radiation, the slit dimension was kept at 3 × 0.45 mm, and 20 mm s^{-1} scanning speed was employed.

System suitability. Parameters including resolution (Rs) and peak symmetry were calculated for both mixtures according to USP guidelines [24].

Construction of calibration curves. Separate aliquots were transferred from the working solution of each drug to prepare solutions of different concentrations. The corresponding chromatographic conditions were applied, and the chromatograms were recorded. The calibration curves were constructed by plotting the recorded peak area $\times 10^{-3}$ against the corresponding concentrations, from which the regression equations were calculated. Calibration curves were constructed in the range of 0.5–2 μg $band^{-1}$ for OFX, 0.5–3.5 μg $band^{-1}$ for DXM, 0.2–1.6 μg $band^{-1}$ for CIP, and 0.6–2 μg $band^{-1}$ for HYD. The calibration curves were constructed using the average of three experiments.

Assay of laboratory-prepared mixtures. Different aliquots of the drugs were accurately transferred from their working solutions and mixed to prepare solutions of different ratios. The chromatographic conditions of both methods were adopted for each laboratory-prepared mixture, and the concentrations of each drug were calculated from the corresponding regression equation. Each concentration was conducted from the average of three experiments.

Application to Pharmaceutical Dosage Forms

Dexaflox® eye drops. Five milliliter of Dexaflox® eye drops was transferred into 10-mL volumetric flask, the volume was completed with methanol to get 1500 μg mL^{-1} of OFX and 500 μg mL^{-1} of DXM. For HPLC method, an appropriate dilution was made with the mobile phase to prepare the working solution to obtain a solution of 30 μg mL^{-1} of OFX and 10 μg mL^{-1} of DXM. For TLC-spectrodensitometric method, an appropriate dilution was made with methanol to prepare the working solution to obtain solutions of 150 μg mL^{-1} of OFX and 50 μg mL^{-1} of DXM.

Ciprocort ® ear drops. One milliliter of Ciprocort ® ear drops was transferred into 10-mL volumetric flask, the volume was completed with methanol to get 230 μg mL^{-1} of CIP and 1 mg mL^{-1} of HYD. For HPLC method, an appropriate dilution was made with the mobile phase to prepare the working solution to obtain solution of 1.15 μg mL^{-1} of CIP and 5 μg mL^{-1} of HYD. For TLC-spectrodensitometric method, an appropriate dilution was made with methanol to prepare the working solution to obtain solution of 23 μg mL^{-1} of CIP and 100 μg mL^{-1} of HYD.

The prepared working solutions of both dosage forms were filtered through 0.45 μm Millipore syringe membrane filter. The corresponding chromatographic conditions were applied for each working solution. Six replicates of each experiment were done. The concentration of each drug was calculated from its corresponding regression equation. The standard addition technique was applied by adding different known concentrations of pure standard drugs to the pharmaceutical formulation before proceeding in the previously mentioned methods.

Results and Discussion

The aim of this work was to develop, validate, and compare between RP-HPLC and TLC-spectrodensitometric methods applied for the determination of two binary mixtures without prior separation.

RP-HPLC Methods

To optimize the RP-HPLC methods, it was necessary to test the effect of different variables. Mobile phases of similar — yet slightly modified — compositions have also been tested, but produced considerably worse results. Different stationary phases, detection wavelengths, and flow rates were also tested. For mixture A, good resolution and linearity were obtained using C_{18} column and acetonitrile: pH-controlled solution (pH 3.3) in the ratio of 45:55 (*v*/*v*) as a mobile phase. Flow rate was kept at 0.6 mL min^{-1}, and detection was done at 240 nm to overcome the problem of the interference of the added preservative (benzalkonium chloride) *(Fig. 2)*. The retention times

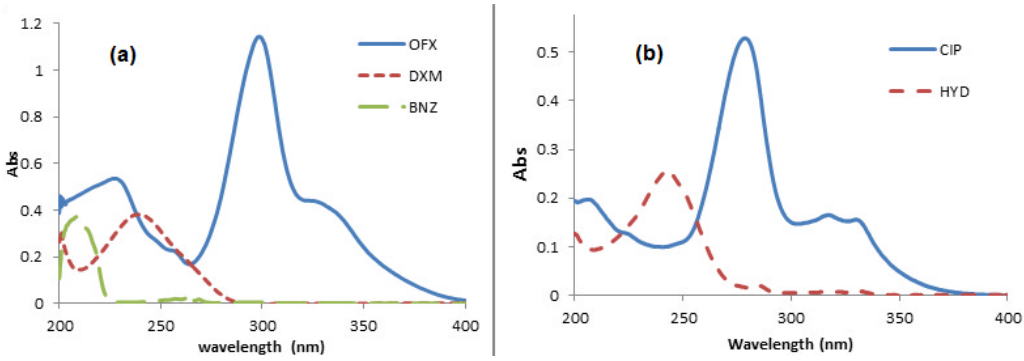

Fig. 2. The zero order UV spectra of (a) 10 μg mL^{-1} of OFX, DXM, and BNZ (benzalkonium chloride); (b) 5 μg mL^{-1} of CIP and HYD

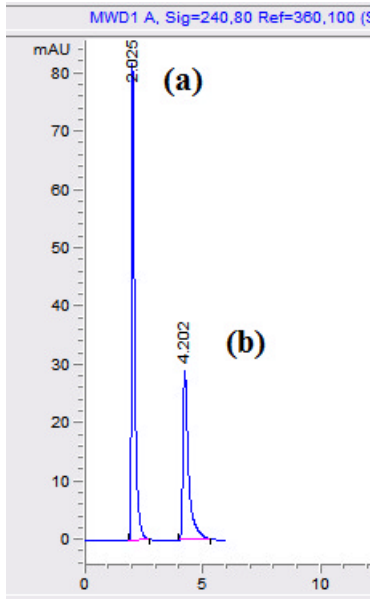

Fig. 3. RP-HPLC chromatogram of 30 μg mL^{-1} (a) OFX and 10 μg mL^{-1}; (b) DXM using C_{18} column, and mobile phase of acetonitrile: pH-controlled solution (pH 3.3) in ratio (45:55 *v*/*v*), flow rate of 0.6 mL min^{-1} at 240 nm

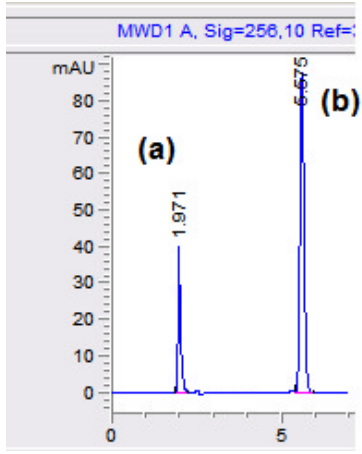

Fig. 4. RP-HPLC chromatogram of 2 μg mL^{-1} (a) CIP and 6 μg mL^{-1}; (b) HYD using C_{18} column and mobile phase of acetonitrile: distilled water: pH-controlled solution (pH 3) in ratio (55:40:5 *v*/*v*/*v*), flow rate of 0.6 mL min^{-1} at 256 nm

were 2.03 and 4.20 for OFX and DXM, respectively *(Fig. 3)*. Although OFX showed a relatively high tailing factor (1.63) as it was reported to have poor symmetrical peak applying different chromatographic conditions [21, 25–

27], this did not affect the reproducibility of the results or the resolution of the two peaks. For mixture B, good resolution and linearity were obtained using C_{18} column and acetonitrile–distilled water–pH-controlled solution (pH 3) in the ratio of 55:40:5 ($v/v/v$) as a mobile phase. Flow rate was kept at 0.6 mL min^{-1}, and detection was done at 256 nm, where both drugs showed good linearity *(Fig. 2)*. The retention times were 1.97 and 5.58 for CIP and HYD, respectively *(Fig. 4)*.

TLC-Spectrodensitometric Methods

This method offers a simple way to quantify the mixtures' components directly on TLC plate by measuring the optical density of the separated bands. The unknown concentrations are determined by being compared to a standard curve from reference materials chromatographed simultaneously under the same condition [28, 29]. Studying the optimum parameters for maximum separation was carried out by trying different developing systems with different ratios, but it was found that the selected developing systems showed the best separation. The developing systems used were chloroform–methanol–triethylamine in the ratio of 35:10:10 ($v/v/v$) for mixture

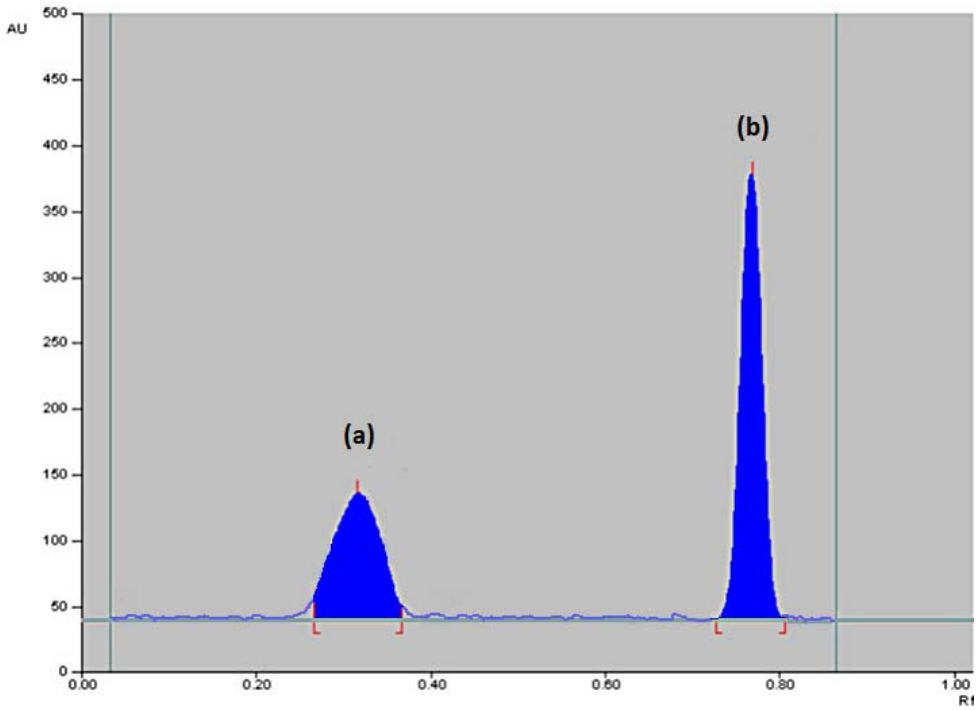

Fig. 5. TLC chromatogram of 1 μg $band^{-1}$ (a) OFX and 1.5 μg $band^{-1}$; (b) DXM using chloroform–methanol–triethylamine (35:10:10 $v/v/v$) as developing system

A and ethyl acetate–hexane–triethylamine in the ratio of 50:25:25 ($v/v/v$) for mixture B. Different scanning wavelengths were checked, but the separated peaks were more sharp and symmetrical with minimum noise upon using 240 nm for mixture A and 243 nm for mixture B. OFX and DXM were separated at R_F 0.35 ± 0.02 and 0.78 ± 0.01, respectively *(Fig. 5)*. CIP and HYD were separated at R_F 0.06 ± 0.02 and 0.66 ± 0.01, respectively *(Fig. 6)*.

System suitability parameters for mixtures A and B, using both methods, were calculated and listed in *Table I*. The assay parameters and validation sheet were listed in *Table II*. The methods were succefully applied to determine the selected drugs in the laboratory-prepared mixtures, and the results are shown in *Tables III* and *V*.

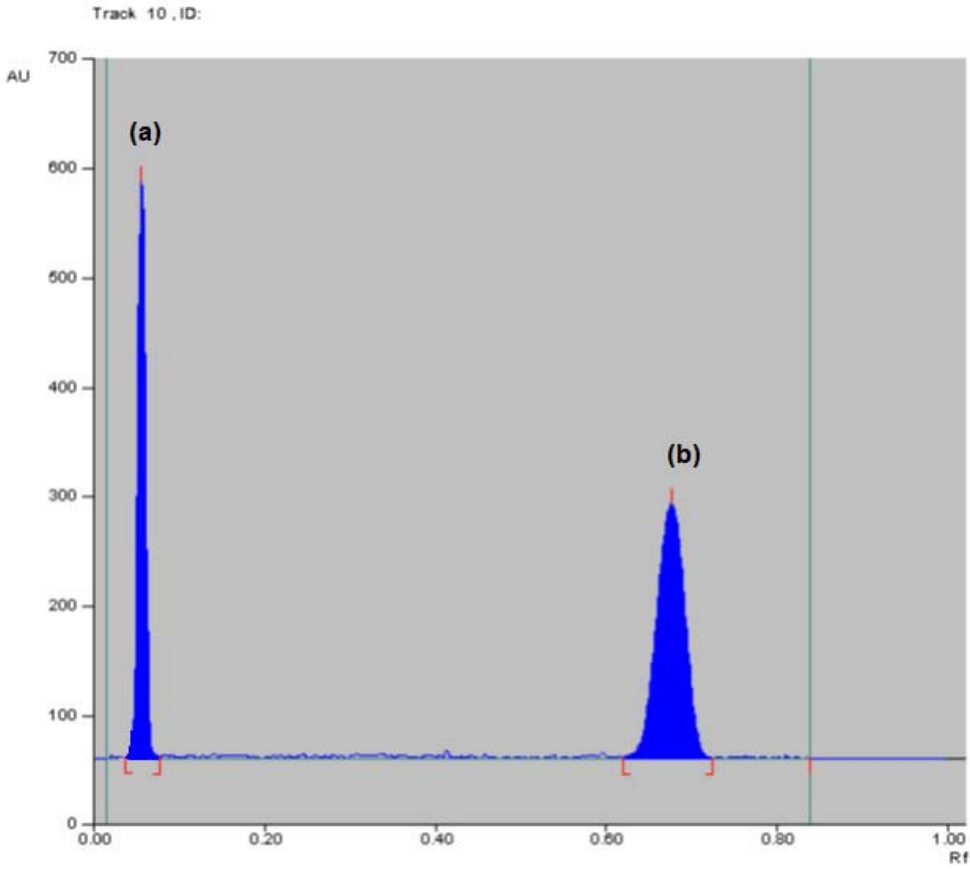

Fig. 6. TLC chromatogram of 1.2 μg band^{-1} (a) CIP and 0.6 μg band^{-1}, (b) HYD using ethyl acetate–hexane–triethylamine (50:25:25 $v/v/v$) as developing system

Table I. Statistical analysis of parameters required for system suitability of HPLC and TLC-spectrodensitometric methods

Parameter	RP-HPLC method				TLC-spectrodensitometric method				Reference value [31]
	OFX	DXM	CIP	HYD	OFX	DXM	CIP	HYD	
t_R (RP-HPLC) R_F (TLC)	2.03	4.20	1.97	5.58	0.35± 0.02	0.78 ± 0.01	0.06± 0.01	0.66± 0.01	$t_R > 1$ (HPLC)
N (column efficiency)	3906	5335	7096	13960					*N* > 2000 Increases with efficiency of the separation
HETP (height equivalent to theoretical plates)	0.004	0.003	0.002	0.001					The smaller the value, the higher the column efficiency
T (tailing factor)	1.63	1.30	1.03	0.89	0.88	1.08	1.13	1.01	*T* < 2 *T* = 1 for symmetric peak
*R*s (experimental resolution)	5.4		8.3		4.5		10.48		*R*s >2

Table II. Assay parameters and validation sheet obtained by applying the RP-HPLC and TLC-spectrodensitometric methods to the binary mixtures A and B

Parameters	RP-HPLC methods				TLC-spectrodensitometric methods			
	OFX	DXM	CIP	HYD	OFX	DXM	CIP	HYD
Calibration range[a]	2.5–45	2.5–50	1–8	1–8	0.5–2	0.5–3.5	0.2–1.6	0.6–2
Slope	0.3676	0.3877	0.8741	0.9405	5.172	3.742	2.969	3.593
Intercept	0.0582	0.0025	0.1235	0.0304	1.105	2.709	2.208	0.2453
Standard error of slope	0.0012	0.0013	0.0051	0.0034	0.0525	0.0382	0.0212	0.0407
Standard error of intercept	0.0292	0.0396	0.0270	0.0169	0.0706	0.0855	0.0214	0.0547
Mean[b]	100.36	100.13	99.89	100.28	99.98	99.93	99.74	99.94
RSD	1.38	1.49	0.61	1.27	1.06	1.18	1.27	1.54
LOD[a]	0.42	0.51	0.12	0.08	0.04	0.09	0.03	0.05
LOQ[a]	1.27	1.56	0.37	0.24	0.13	0.27	0.09	0.14
Accuracy[c]	100.91/ 1.06	100.84/ 0.95	100.32/ 0.78	99.89/ 0.34	100.72/ 1.41	101.35/ 0.60	100.50/ 1.00	100.21.59
Correlation coefficient (*r*)	0.9999	0.9999	0.9999	0.9999	0.9997	0.9997	0.9998	0.9997
R^2	0.9999	0.9999	0.9998	0.9999	0.9995	0.9994	0.9997	0.9994
Intra-day precision[c]	100.99/ 0.74	101.02/ 0.66	100.37/ 0.19	99.86/ 0.46	100.53/ 1.35	99.76/ 0.86	100.29/ 0.65	99.78/ 1.15
Inter-day precision[c]	99.37/ 0.95	100.15/ 0.87	99.89/ 0.40	99.83/ 0.45	100.09/ 1.07	100.23/ 1.07	100.20/ 1.01	99.29/ 0.76
Robustness[c]	99.11/ 0.53	100.03/ 0.51	100.32/ 0.42	99.95/ 0.61	100.79/ 1.16	101.44/ 1.54	100.94/ 1.28	100.43/ 1.73

[a]RP-HPLC methods: in (μg mL^{-1}); TLC-spectrodensitometric methods: in μg $band^{-1}$.
[b]Average of three experiments.
[c]Mean value/RSD of three samples.

Table III. Determination of OFX and DXM in laboratory-prepared mixtures by the RP-HPLC and TLC-spectrodensitometric methods

Mixture no.	Ratio	RP-HPLC method						TLC-spectrodensitometric method					
		OFX			DXM			OFX			DXM		
		Taken (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)	Taken (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)	Taken (μg band^{-1})	Found[a] (μg band^{-1})	Recovery (%)	Taken, (μg band^{-1})	Found[a] (μg band^{-1})	Recovery, %
1	1:1	25	25.29	101.16	25	25.09	100.36	1	1.01	101.00	1	0.98	98.18
2	1:2	25	25.12	100.48	50	50.22	100.44	1	1.01	101.00	2	2.02	101.00
3	1:3	10	10.2	100.20	30	30.00	100.00	1	1.02	102.00	3	3.01	100.33
4	2:1	40	39.86	99.65	20	20.18	100.90	2	1.99	99.50	1	1.01	101.00
5	2:3	20	19.76	98.80	30	30.00	100.00	2	1.97	98.53	3	3.04	101.33
6	3:1[b]	30	29.87	99.57	10	10.01	100.10	1.5	1.50	100.06	0.5	0.50	100.00
7	3:2	30	29.98	99.93	20	19.90	99.50	1.5	1.49	99.33	1	1.02	102.00
Mean ± SD				99.97 ± 0.75			100.19 ± 0.44			100.20 ± 1.19		100.55 ± 1.23	

[a]Average of three experiments.
[b]Ratio present in Dexaflox® eye drops.

Table IV. Application of standard addition technique to the analysis of OFX and DXM in Dexaflox® eye drops by the RP-HPLC and TLC-spectrodensitometric methods compared to reported method [21]

Dexaflox® eye drops Batch no:LB060	RP-HPLC method					TLC-spectrodensitometric method					Reported method [21]
	Claimed (μg mL^{-1})	Found (μg mL^{-1})	Recovery%[a]	Pure added (μg mL^{-1})	Recovery%[b]	Claimed (μg band^{-1})	Found (μg band^{-1})	Recovery%[a]	Pure added (μg.band^{-1})	Recovery%[b]	Recovery%[a]
OFX	30	30.60	102.00 ± 0.82	2.5	100.96	1.5	1.53	101.39 ± 0.82	0.5	100.96	101.48 ± 1.04
				5	100.43				0.75	100.43	
				10	100.13				0.85	100.13	
				15	99.99				1.0	99.99	
Mean ± SD					100.18 ±0.55					100.38 ±0.43	
DXM	10	9.95	99.49 ± 0.52	5	99.68	0.5	0.50	100.76 ± 1.17	0.5	99.68	99.69 ± 0.63
				10	100.83				1.0	100.83	
				15	99.89				1.5	99.89	
				20	100.50				2.0	100.50	
Mean ± SD					100.43 ±0.72					100.22 ±0.53	

[a]Average of six experiments.
[b]Average of three experiments.

Table V. Determination of CIP and HYD in laboratory-prepared mixtures by the RP-HPLC and TLC-spectrodensitometric methods

Mixture no.	Ratio	RP-HPLC method						TLC-spectrodensitometric method					
		CIP			HYD			CIP			HYD		
		Taken (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)	Taken (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)	Taken (μg band^{-1})	Found[a] (μg band^{-1})	Recovery (%)	Taken (μg band^{-1})	Found[a] (μg band^{-1})	Recovery (%)
1	1:1	2	2.01	100.54	2	1.99	99.54	0.8	0.803	100.32	0.8	0.798	99.77
2	1:2	2	1.99	99.69	4	4.03	100.69	0.4	0.404	100.98	0.8	0.797	99.65
3	1:3	1	1.01	100.80	3	2.99	99.66	0.2	0.203	101.43	1.6	1.610	100.63
4	1:5	1	1.01	101.18	5	5.00	100.01	0.2	0.201	100.32	1	1.009	100.93
5	2:1	4	4.04	100.93	2	2.01	100.59	1.2	1.197	99.76	0.6	0.608	101.32
6	2:3	2	1.99	99.64	3	2.99	99.90	0.6	0.598	99.65	0.9	0.900	100.02
7	3:2	3	2.99	99.79	1	1.00	99.72	0.9	0.907	100.82	0.6	0.599	99.84
Mean ± SD				100.38 ± 0.60			100.11 ± 0.50			100.47 ± 0.65			100.31 ± 0.61

[a]Average of three experiments.

Application to Pharmaceutical Dosage Forms

The suggested HPLC and TLC-spectrodensitometric methods were valid and applicable for the analysis of OFX and DXM in Dexaflox® eye drops, CIP and HYD in Ciprocort® ear drops. The validity of the proposed methods was further assessed by applying the standard addition technique,

Table VI. Application of standard addition technique to the analysis of CIP and HYD in Ciprocort ® ear drops by the RP-HPLC and TLC-spectrodensitometric methods compared to manufacturer method [29]

Ciprocort® ear drops Batch no: 1601001	RP-HPLC method					TLC-spectrodensitometric method					Manufacturer method [29] [c]
	Claimed (μg mL^{-1})	Found (μg mL^{-1})	Recovery %[a]	Pure added (μg mL^{-1})	Recovery %[b]	Claimed (μg band^{-1})	Found (μg band^{-1})	Recovery %[a]	Pure added (μg band^{-1})	Recovery %[b]	Recovery %[a]
CIP	1.15	1.16	101.20 ± 0.48	2	2.01	0.230	0.233	101.48 ± 0.51	0.5	100.96	101.66 ± 0.81
				3	3.04				0.75	100.43	
				4	4.01				0.85	100.13	
				5	4.98				1.0	99.99	
Mean ± SD					100.40 ± 0.73					100.15 ±0.41	
HYD	5	4.91	98.14 ± 0.36	1	1.00	1.0	0.982	98.25 ± 0.34	0.2	0.201	98.01 ± 0.64
				1.5	1.50				0.4	0.399	
				2	2.00				0.6	0.601	
				2.5	2.53				0.8	0.803	
Mean ± SD					100.27 ± 0.55					100.25 ±0.41	

[a]Average of six experiments.

[b]Average of three experiments.

[c] Manufacturer method [29] is HPLC using RP C18 and mobile phase water–methanol (55:45) pH 2.5 with o-H_3PO_4, flow rate 1.5 mL/min, detection at 240 nm.

which showed accurate results. The results for mixture A were compared with that of the reported method [21], and the results for mixture B were compared with that of the manufacturer method [30], as shown in *Tables IV* and *VI*, respectively. The results confirm the suitability of the proposed methods for the routine determination of these components in their combined formulations.

Methods Validation

Method validation was performed according to ICH guidelines [31] for all the proposed methods as follows:

Range and Linearity

The linearity of the proposed methods was evaluated by processing the different calibration curves on 3 different days. The RP-HPLC methods showed higher correlation coefficients (r = 0.9999) than TLC-spectrodensitometric methods (r = 0.9997). The calibration curves were constructed within concentration ranges that were selected on the basis of the anticipated drugs concentration during the assay of the dosage form. For RP-HPLC methods, calibration curves were constructed in the range of 2.5–45 μg mL^{-1} for OFX, 2.5–50 μg mL^{-1} for DXM, and 1–8 μg mL^{-1} for both CIP and HYD. For TLC-spectrodensitometric methods, calibration curves were constructed in the range of 0.5–2 μg $band^{-1}$ for OFX, 0.5–3.5 μg $band^{-1}$ for DXM, 0.2–1.6 μg $band^{-1}$ for CIP, and 0.6–2 μg $band^{-1}$ for HYD *(Table V)*. The corresponding assay parameters and validation sheet for the proposed methods were listed in *Table II*.

Limits of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated for each drug using the proposed methods with a ratio of 3.3 and 10 of standard deviations of the blank, respectively, and the slope of the calibration line, as shown in *Table II*.

Accuracy

To study the accuracy of the proposed methods, procedures under linearity were repeated three times for the determination of different blind concentrations of pure drugs. The accuracy expressed as percentage recoveries/RSD is shown in *Table II*. The interference of excipients in the pharmaceutical formulations was studied by applying standard addition method to

the pharmaceutical formulation. Good accuracy proved that the excipients in pharmaceutical formulations did not interfere in the analysis of these compounds, as shown in *Tables IV* and *VI*.

Precision

The precision of the proposed methods, expressed as RSD, was determined by the analysis of three different concentrations of pure drugs within the linearity range. The intra-day precision was assessed from the results of three replicate analyses of three pure drugs samples on a single day. The inter-day precision was determined from the same samples analyzed on 3 consecutive days. Comparing the results of the proposed methods for each mixture, it was found that RP-HPLC methods were more precise than TLC-spectrodensitometric methods (lower RSD). The results are illustrated in *Table II*.

Selectivity

Selectivity of the proposed methods was achieved by the analysis of different laboratory-prepared mixtures of A (OFX and DXM) and B (CIP and HYD) within the linearity range. Satisfactory results were shown in *Tables III* and *V*.

Robustness

The robustness of the proposed methods was investigated by the analysis of samples under a variety of experimental conditions. For RP-HPLC methods, small changes in the pH (±0.5) and small changes in proportions of acetonitrile by up to ±2% were introduced to the mobile phases. A slight change in the retention time and peak parameters was observed, however, the peak areas were conserved. For TLC-spectrodensitometric methods, small changes in proportions of chloroform (mixture A) and ethyl acetate (mixture B) by up to ±2% were introduced to the developing systems. R_F values and peak symmetry were slightly changed; however, the peak areas were conserved. The effect of robustness was more observed in the TLC methods (higher RSD) as listed in *Table II*, which proved that RP-HPLC methods were more robust upon changing the experimental conditions

Statistical Analysis

Results obtained by the proposed methods for the determination of pure samples of OFX, DXM, CIP, and HYD were statistically compared to those

obtained by the official methods [2]. The values of the calculated *t* and *F* were less than the corresponding tabulated ones, which revealed that there was no significant difference with respect to accuracy and precision between the proposed methods and the official ones as shown in *Table VII*.

Table VII. Statistical comparison between the results obtained by the proposed methods and the official BP methods [2] for the determination of OFX, DXM, CIP, and HYD in pure powder form

Parameters	RP-HPLC methods				TLC-spectrodensitometric methods				Official BP methods [2][a]			
	OFX	DXM	CIP	HYD	OFX	DXM	CIP	HYD	OFX	DXM	CIP	HYD
Mean	100.36	100.13	99.89	100.28	99.98	99.93	99.74	99.94	100.21	101.82	99.86	100.52
RSD	1.38	1.49	0.61	1.27	1.06	1.18	1.27	1.54	0.95	1.09	0.85	0.64
No. of experiments	7	7	7	7	7	7	7	7	5	5	5	5
Student's *t*-test (1.81)[b]	1.67	0.67	1.09	0.87	1.56	0.45	1.32	1.01				
F test (4.53)[a]	1.78	3.69	2.03	2.44	1.05	3.57	1.15	3.17				

[a]BP methods for OFX is a non-aqueous potentiometric titrimetric method, for CIP is HPLC method, while for DXM and HYD are absorbance methods.

[b]Figures between parentheses represent the corresponding tabulated values of *t* and *F* at $P = 0.05$.

Conclusion

Comparing the RP-HPLC and TLC-spectrodensitometric methods for analysis of both mixtures, it was found that the proposed methods provided simple, accurate, sensitive, and selective quantitative analysis of both mixtures in bulk powder, laboratory-prepared mixtures, and dosage forms. The TLC-spectrodensitometric methods had the advantages, over the reported and proposed RP-HPLC methods, of being simpler (simple developing systems with no pH adjustments), sensitive and economic, as it saves cost (inexpensive apparatus and solvents), and time, as up to 20 samples could be applied to a single plate and analyzed per one development. The proposed RP-HPLC methods had the advantage of being more precise and robust than the proposed TLC-spectrodensitometric methods. The proposed RP-HPLC applied a simple isocratic mobile phase, unlike the reported methods [19, 20, 22, 23] through which the conditions, the mobile phase ratio, and composition seemed rather critical; so the robustness of the reported methods could therefore be significantly affected. The proposed RP-HPLC method differed from the reported method [21] through using different conditions (shorter column, lower flow rate, and modified mobile phase),

which lead to consuming smaller amounts of solvents on the large scale (quality control laboratories) and saving the lifetime of the column used, but yet the same retention time, symmetry, and resolution of the peaks were reserved.

References

[1] Sweetman, SC (ed.) Martindale: The Complete Drug Reference. Pharmaceutical Press, London, 2005
[2] British Pharmacopoeia, The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA)-© Crown Copyright, 2009
[3] A.I. Drakopoulos, and P.C. Ioannou, Anal. Chim. Acta, **354**, 197–204 (1997)
[4] G.H. Ragab, and A.S. Amin, Spectrochim. Acta, Part A, **60**, 973–978 (2004)
[5] H. Hopkala and D. Kowalczuk, Acta Pol. Pharm., **57**, 3–13 (2000)
[6] J.D. Smet, K. Bousserya, K. Colpaertb, P. De Suttera, P. De Paepec, J. Decruyenaereb, and J.V. Bocxlaera, J. Chromatogr., B, **877**, 961–967 (2009)
[7] V.F. Samanidou, C. Demetriou, and I.N. Papadoyannis, Anal. Bioanal. Chem., **375**, 623–629 (2003)
[8] R. Singh, M. Maithani, S.K. Saraf, S. Saraf, and R.C. Gupta, Eurasian J. Anal. Chem. **4**, 161–167, (2009)
[9] Y.L. Feng, and C. Dong C, J. Chromatogr. Sci., **42**, 474–477 (2004)
[10] S. Han-wen, H. Pan, L. Yun-kai, and L. Shu-xuan, J. Chromatogr., B, **852**, 145–151 (2007)
[11] F.A. El-Yazbi, H.H. Hammud, and S.A. Assi, Anal. Chim. Acta, **580**, 39–46 (2006)
[12] J.M. Lemus Gallego and J. P. Arroyo, Anal. Chim. Acta, **460**, 85–97 (2002)
[13] Q. Chen, D. Zielinski, J. Chen, A. Koski, D. Werst, and S. Nowak, J. Pharm. Biomed. Anal., **48**, 732–738 (2008)
[14] Y. Xiong, K.P. Xiao, and A.M. Rustum, J. Pharm. Biomed. Anal., **49**, 646–654 (2009)
[15] E. Grippa, L. Santini, G. Castellano, M.T. Gatto, M.G. Leone, and L. Saso, J. Chromatogr., B, **738**, 17–25 (2000)
[16] M. Pendela, G. Kahsay, I. Baekelandt, A.V. Schepdael, and E. Adams, J. Pharm. Biomed. Anal., **56**, 641–644 (2011)
[17] V. Baeyens, E. Varesio, J.L. Veuthey, and R. Gurny, J. Chromatogr., B, **692**, 222–226 (1997)
[18] H. Sirén, T. Seppänen-Laakso, M. Orešič, J. Chromatogr., B, **871**, 375–382 (2008)
[19] T. Ruiwei, M. Mingxiu, and L. Genghao, West China J. Pharm. Sci., **4**, 45–46 (1999)
[20] L. Wei, G. Hua, and Z. Fantao, China Pharm., **10**, 1 (2005)
[21] K. Sireesha, and K. Prakash, Int. J. Pharm. Pharm. Sci., **4**, 415–418 (2012)
[22] W. Chun-Lan, L. Shi-Cai, and L. Lan-Ju, Chinese J. Pharm., **1** (2004)
[23] L. Li, Determination of Ciprofloxacin and Hydrocortisone in Compound Ciprofloxacin Ear Drops by HPLC. Chinese Pharmaceutical Affairs, **3** (2008)
[24] United States Pharmacopoeia Commission, United States Pharmacopeia—National formulary. United States Pharmacopeial Inc., 2280–2282. 2004
[25] V.P. Ranea, and D.B. Shindea, Eurasian J. Anal. Chem., **4**, 304–310 (2009)
[26] A.S. Amin, H.A. Dessouki, and IA Agwa, Arab J. Chem., **4**, 249–257 (2011)
[27] K.S. Khandagle, SV. Gandhi, P.B. Deshpande, and N.V. Gaikwad, Int. J. Pharm. Pharm. Sci., 3(1), 46–48 (2011)

[28] N.E. Wagieh, M.A. Hegazy, M. Abdelkawy, and E.A. Abdelaleem, Talanta, **80**, 2007–2015 (2010)
[29] Y.S. El-Saharty, N.A. El-Ragehy, H.M. Abdel-Monem, and M.I. Abdel-Kawy, JAR, **1**, 71–78 (2010)
[30] European Egyptian Pharmaceutical Industries (EEPI) Quality Control Department, Test Method for Determination of Ciprocort Ear Drops
[31] International Conference on Harmonization (ICH), Q2B Validation of Analytical Procedures: Methodology, 62, US FDA, Federal Register, 1997

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