Thin-Layer Chromatographic Enantioseparation of Ofloxacin and Zopiclone using Hydroxy-Propyl-Beta-Cyclodextrin as Chiral Selector and Thermodynamic Studies of Complexation

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Key Words

Eszopiclone
Levofloxacin
Enantiomeric purity
Densitometry–TLC
Chiral mobile phase additive
Thermodynamic study

Summary

A novel economic thin-layer chromatographic procedure for stereoselective separation of racemic mixtures of each of zopiclone and ofloxacin, and determination of their enantiomers: eszopiclone, (+)-(*S*)-zopiclone, and levofloxacin, (−)-(*S*)-ofloxacin, was described. The method was based on using normal plates and hydroxy propyl-β-cyclodextrin (HP-β-CD) as chiral mobile phase additive (CMPA). The spots were detected under UV lamp 254 nm, followed by densitometric measurements at 304 and 330 nm for (+)-(*S*)-zopiclone and (−)-(*S*)-ofloxacin, respectively. The mobile phase enabling successful resolution of the drugs was ethanol–acetonitrile–glacial acetic acid–diethylamine–distilled water containing 0.5% HP-β-CD (4:2:3:1:1, by volume), pH 4, for zopiclone and ethanol–acetonitrile–glacial glacial acetic acid–diethylamine–distilled water containing 0.3% HP-β-CD (4:4:3:2:1 by volume), pH 4.5, for ofloxacin at 25 ± 2°C. All variables affecting the resolution, such as concentration of different chiral selectors, temperature, and pH, were investigated, and the conditions were optimized. Furthermore, some thermodynamic parameters were calculated. The procedure provided a linear response over the concentration range of 1–4 and 2–7 μg spot^{-1} for determination of pure active isomers, (+)-(*S*)-zopiclone and (−)-(*S*)-ofloxacin, respectively, with acceptable precision (relative standard deviation [% RSD] <2.0). The developed method was validated and proved to be robust. The proposed method was found to be selective and accurate for the identification and quantitative determination of enantiomeric purity of the two active isomers in their drug substances and drug products.

1 Introduction

Zopiclone, (RS)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-5-yl-4-methylpiperazine-1-carboxylate, is a nonbenzodiazepine hypnotic agent used in the treatment of insomnia. It has two enantiomeric isomers, which are metabolized at different rates [1]. Eszopiclone, (+)-(*S*)-zopiclone **(Figure 1),** is pharmacologically more active and exhibits fifty times higher affinity towards the benzodiazepine receptor binding site than the (−)-(*R*)-enantiomer [2]. Resolution of zopiclone enantiomers was reported by different methods as biphasic recognition chiral extraction [3]. Enantiomeric separation of zopiclone and its metabolites, and degradation products by high-performance liquid chromatography (HPLC) were reported [4–7]. Ofloxacin, having the chemical name (RS)-9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo7H-pyrido [1,2,3-de]-1,4-benzooxazine-6-carboxylic acid, belongs to the quinoline class of antibiotics [1]. Pharmaceutical research has shown that the antibacterial activity of the levo-enantiomer, (−)-(*S*)-ofloxacin (Figure 1), is 8–128 times higher than that of (+)-(*R*)-ofloxacin and twice as potent as that of the racemate [8, 9]. Chiral separation of ofloxacin in biological fluid has been achieved by HPLC using chiral mobile phase additive [10, 11]. A number of capillary electrophoresis (CE) methods have been proposed for chiral separation of ofloxacin enantiomers, using different chiral selectors: cyclodextrins (CDs) [12–14], vancomycin [15], bovine serum albumin [16, 17], or combination of CD and D-phenylalanine, and zinc sulphate [18].

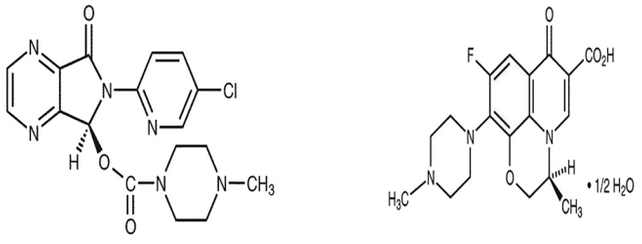

Figure 1

The chemical structures of eszopiclone (A) and levofloxacin (B).

Since these enantiomers exhibit different biological, pharmacological, and toxicological properties [19], therefore, the aim of this paper is to develop thin-layer chromatography (TLC) method for the separation of racemic mixture of zopiclone and

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ofloxacin followed by determination of active enantiomers in their drug substances and products using hydroxy propyl-beta-cyclodextrin (HP-β-CD) as chiral mobile phase additive.

2 Experimental

2.1 Apparatus

Shimadzu dual wavelength flying spot densitometer Model CS-9301 PC (Tokyo, Japan) and Hamilton microsyringe (25 µL capacity) were used for spotting. Normal TLC aluminum plates (10 × 20 cm^2), with 0.2 mm thickness silica gel F_{254} (Macherey-Nagel, Germany) were used. UV short-wavelength (254 nm, 350 nm) lamp (Desaga, Germany) was used for detection. Magnetic stirrer (Accumax, New Delhi, India) was applied.

2.2 Pure and Market Samples

Zopiclone was supplied by Amoun Pharmaceutical Industries Company (Cairo, Egypt), and its purity was labelled to be 100.84 ± 1.944 [20]. Eszopiclone was supplied by Medizen Pharmaceutical Industries (Alexandria, Egypt), and its purity was labelled to be 98.76 ± 0.861. Ofloxacin and levofloxacin were supplied by Sanofi Aventis (Cairo, Egypt), and their purities were labeled to be 99.08 ± 0.255 and 100.67 ± 1.19, respectively [20]. Hypnor® tablet, labelled to contain 7.5 mg per tablet of zopiclone, was manufactured by Amoun Pharmaceutical Industries Company (Cairo, Egypt) (Batch No. 21559). Oflicin eye drops, labelled to contain 300 mg/100 mL of ofloxacin, was manufactured by Memphis Company (Cairo, Egypt) (Batch No. OFX258). Otoxin ear drops, labelled to contain 3 mg mL^{-1} of ofloxacin (Batch No. 963547), and Venaxan intravenous infusion, labelled to contain 500 mg levofloxacin/100 mL (Batch No. 22895), were both manufactured by Sedico Company (Cairo, Egypt). Levoflox intravenous infusion, labelled to contain 500 mg levofloxacin/100 mL, was manufactured by Memphis Company (Cairo, Egypt) (Batch No. 26452). All were purchased from the local market.

2.3 Chemicals and Reagents

All chemicals and reagents used were of analytical grade. Ethanol (absolute; Fischer Scientific, UK), acetonitrile and methanol (Labscan, Ireland), diethyl amine (Merck, Darmstadt, Germany), hydroxy propyl-beta-cyclodextrin (HP-β-CD) (Fluka Chemicals Ltd., UK), glacial acetic acid (Fischer Scientific, UK), and double distilled water were used.

2.4 Standard Solutions

An accurately weighed amount – 250 mg of each of eszopiclone, levofloxacin (active isomers), and zopiclone and ofloxacin (racemic forms) – was transferred into separate 25-mL volumetric flasks and dissolved in 20 mL methanol, and then the volumes were completed with methanol (10 mg mL^{-1}).

2.5 Working Standard Solutions

Working standards solutions were prepared in concentration ranges of 200–800 µg mL^{-1} zopiclone, 100–400 µg mL^{-1} eszopi-

Table 1

Optimization parameters for chiral separation of racemic mixtures of zopiclone and ofloxacin.

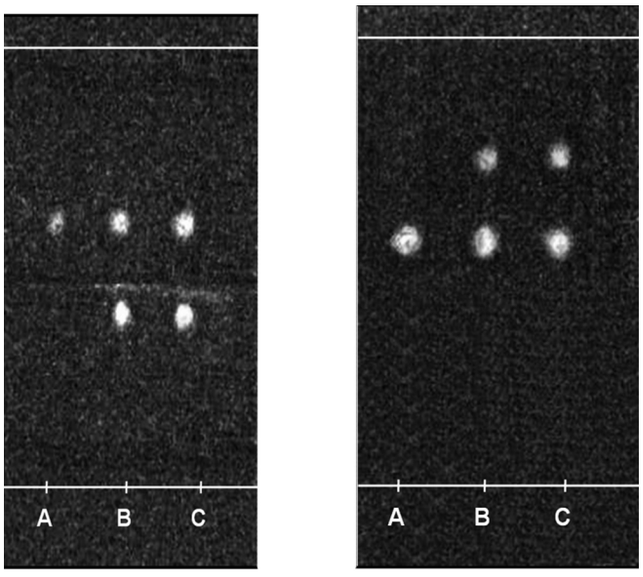
Parameters	Modification		hR_{F1}[a] S-isomer		hR_{F2}[a] R-isomer		α[b]	
	Zopiclone	Ofloxacin	Zopiclone	Ofloxacin	Zopiclone	Ofloxacin	Zopiclone	Ofloxacin
Chiral selector	0.3%	0.1%				NR[c]		
concentration	0.4%	0.2%				RT[d]		
(HP-β-CD)	0.5%	0.3%	55	33	40	16	1.83	2.58
	0.6%	0.4%	54	33	45	20	1.43	1.97
	0.7%	0.5%	50	32	46	25	1.17	1.41
pH	2	2				NR[c]		
	3	3				NR[c]		
	4	4.5	55	33	40	16	1.83	2.58
	5	5	53	33	47	22	1.27	1.74
	6	6	53	31	48	24	1.22	1.42
	7	7	55	RT	50	RT	1.22	1.97
Temperature	5	5	54	82	41	58	1.70	3.31
(± 2°C)	10	10	51	72	38	45	1.61	3.15
	15	15	48	61	37	38	1.57	2.55
	20	20	45	54	38	37	1.33	2.00
	25	25	39	33	33	26	1.29	1.40

[a] $hR_F = R_F \times 100$

[b] $\alpha = [(1/R_{F1}) - 1] / [(1/R_{F2}) - 1]$

[c] NR, no resolution

[d] RT, resolution with tailing



a) b)

Figure 2

Photographs of chromatograms of zopiclone and ofloxacin. a) (*R*,*S*)-Zopiclone using ethanol–acetonitrile–glacial acetic acid–diethylamine–distilled water containing 50 mg HP-β-CD (4:2:3:1:1, by volume) as a developing system. A: pure eszopiclone; B: (*R*,*S*)-zopiclone in drug substance, lower spot is the *R*-isomer (inactive form) and upper spot is the *S*-isomer (active form); C: (±)-zopiclone in Hypnor tablet. b) (±)-Ofloxacin using ethanol–acetonitrile–glacial glacial acetic acid–diethylamine–distilled water containing 30 mg HP-β-CD (4:4:3:2:1 by volume as a developing system. A: pure levofloxacin; B: (±)-ofloxacin in drug substance, lower spot is the levo isomer (active form) and upper spot is the dextro isomer (inactive form); C: (±)-ofloxacin in eye and ear drop.

clone, 400–1400 μg mL^{-1} ofloxacin, and 200–700 μg mL^{-1} levofloxacin by appropriate dilution of standard solution with methanol.

2.6 Chromatographic Conditions

Ten microliters of each pure active isomer and racemic mixtures of working solutions were applied as separate compact spots 20 mm apart from each other and 20 mm from the bottom of the TLC plate. The chromatographic chambers (12 × 12 × 24 cm) were previously saturated for 20 min. The plates were developed at 25 ± 2°C for 30 min using solvent system of ethanol–acetonitrile–glacial acetic acid–diethylamine–distilled water containing 50 mg HP-β-CD (4:2:3:1:1, by volume), pH 4, for zopiclone and

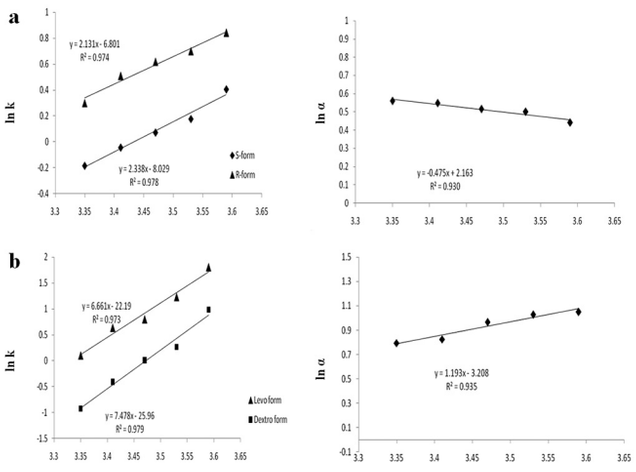


Figure 3

Van't Hoff plots to estimate the thermodynamic properties for enantioseparation of zopiclone (a) and ofloxacin (b).

ethanol–acetonitrile–glacial glacial acetic acid–diethylamine–distilled water containing 30 mg HP-β-CD (4:4:3:2:1, by volume), pH 4.5, for ofloxacin. The plates were dried at room temperature, visualized under UV lamp (254 nm), and scanned at 304 nm and 330 nm for eszopiclone and levofloxacin, respectively, under the following instrumental conditions: photomode: reflection, scan mode: zigzag, swing width: 12, and result output: chromatogram and area under the peak.

2.7 Method Validation [21]

The methods were validated in accordance with the International Conference on Harmonization (ICH) guidelines by documenting their linearity, accuracy, precision, and limits of detection (LOD) and quantification (LOQ).

2.8 Application to Pharmaceutical Dosage Forms

2.8.1 Hypnor Tablet

Twenty tablets were weighed and ground to a fine powder. An adequately weighted amount equivalent to 100 mg of zopiclone was transferred to a 100-mL volumetric flask, and 50 mL acetonitrile was added. The contents of the flask were sonicated for 20 min to affect complete dissolution of zopiclone and filtered. The filtrate was quantitatively transferred to 100-mL volumetric flask and completed to the volume with acetonitrile. Then the proposed procedure was followed.

Table 2

Thermodynamic data for zopiclone and ofloxacin enantiomers determined at different temperatures using hydroxy propyl β-CD as chiral selector.

Compound	$\Delta H°$ (kJ/mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})	R^2	$\Delta\Delta H°$	$\Delta\Delta S°$	R^2	$\Delta\Delta G°$ (kJ/mol^{-1})
S-zopiclone	−19.407 ± 0.123	−66.636 ± 0.821	0.978	−3.949 ± 0.990	−9.437 ± 1.033	0.931	−2.816
R-zopiclone	−17.677 ± 0.521	−56.417 ± 1.125	0.972				
S-ofloxacin	−55.386 ± 0.368	−22.189 ± 1.085	0.973	−9.919 ± 0.487	−26.674 ± 1.265	0.935	−7.938
R-ofloxacin	−62.179 ± 1.115	−25.959 ± 0.744	0.979				

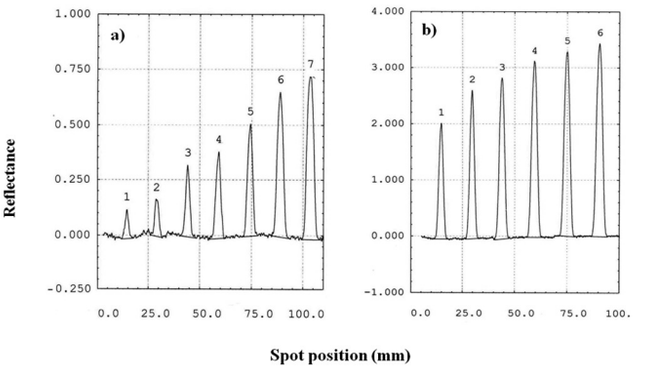


Figure 4

Densitograms of different concentrations of 1.0–4.0 µg spot^{-1} at 304 nm and 2.0–7.0 µg spot^{-1} at 330 nm for eszopiclone (a) and levofloxacin (b), respectively.

2.8.2 Oflicin Eye Drops and Otoxin Ear Drops

The content of 5 plastic bottles was mixed, and an accurately measured volume of each dosage form equivalent to 20.0 mg of ofloxacin was directly transferred into a 10-mL volumetric flask and diluted with methanol. Then the proposed procedure was followed.

2.8.3 Venaxan Vial and Levoflox Solution for Intravenous Infusion

An accurately measured volume equivalent to 100 mg levofloxacin of each Venaxan vial or Levoflox solution for intravenous infusion was transferred into a 100-mL volumetric flask and diluted to the mark with methanol to obtain solutions of 1 mg mL^{-1}, and then the general procedure was followed.

Table 3

Validation and regression equations parameters for the proposed method for the determination of eszopiclone and levofloxacin drug substances.

Parameters	Eszopiclone	Levofloxacin
Linearity range (µg spot^{-1})	1–4	2–7
LOD (µg spot^{-1})	0.154	0.351
LOQ (µg spot^{-1})	0.466	1.095
– Accuracy (n = 6)		
Mean ± % RSD[a)]	101.50 ± 0.372	101.79 ± 0.360
Mean ± % RSD[b)]	47.92 ± 0.893	47.82 ± 1.434
Mean ± % RSD[b)]		48.14 ± 1.058
– Precision (n = 9)		
Instrumental repeatability		
R_F	0.512	0.651
Peak areas	0.670	0.691
Method repeatability		
R_F	0.266	0.318
Peak areas	0.146	0.164
Inter-day precision		
R_F	0.298	0.595
Peak areas	0.134	0.269
– Regression equation		
– Slope	1354.07	1278.2
SE of the slope	12.309	18.31
Confidence limit of the slope[c)]	1322.429–1385.714	1227.35–1329.04
– Intercept	830.535	2663
SE of the intercept	33.144	88.140
Confidence limit of the intercept[c)]	745.335–915.736	2418.548–2907.985
– Correlation coefficient (r)	0.9997	0.9995
SE of estimation	32.568	76.606

[a)]Drug substances

[b)]Pharmaceutical formulations

[c)]95% Confidence limit

Table 4a

Accuracy of the proposed TLC–densitometric method for the determination of eszopiclone in drug substance and drug product.

Concentration (μg spot^{-1})	Drug substance (pure isomer)			Pharmaceutical formulation (Hypnor® tablet 7.5 mg)		
	Found conc. (μg spot^{-1})	Recovery[a)] (%)	% RSD	Found conc. (μg spot^{-1})	Recovery[a)] (%) of claimed amount	% RSD
1	1.01	101.00	0.648	0.96	48.00	0.972
1.5	1.53	101.33	1.006	1.43	47.66	0.807
2	2.04	101.50	0.975	1.93	48.25	0.853
3	3.11	102.00	1.233	2.84	47.33	0.922
3.5	3.56	101.71	0.709	3.38	48.37	0.714
Mean ± % RSD		101.50 ± 0.372			47.92 ± 0.893	

[a)]Average of four different determinations

3 Results and Discussion

Almost half of the drugs in use are chiral. It is well known that the pharmacological effect is restricted in most of the cases to one of the enantiomers [22]. Nevertheless, only about 25% of drugs are administered as pure enantiomers. The enantiomers can differ in absorption, distribution, protein binding, and affinity to the receptor [23]. Furthermore, the metabolic pathways can differ. Thus, in recent years, there has been an increasing demand for the separation of enantiomers with chromatographic technique. This is expressed by a rapidly growing number of publications and innovation in this field, mostly concentrated on HPLC and gas chromatography (GC). Besides this technique, which requires expensive apparatus, there is a demand for a simple, economic, and fast method for the determination of enantiomeric composition. Here, TLC, with an enantioselective stationary phase, is the method of choice [24]. This is particularly noticeable with enantiomeric separations where some of the strict criteria essential for resolution in HPLC are not so necessary in TLC; thus, a wide range of mobile phases and detection reagents can be used. The plates are quickly and easily prepared and, for the most part, have good stability. Little or no sample pretreatment is required before chromatography. As modern spectrodensitometers are capable of in-situ scanning of developed TLC/high-performance thin-layer chromatography (HPTLC) plates by UV absorption, reflectance, fluorescence, and fluorescence quenching, the detection range is extensive. This can be further widened by reaction of the sample spots or bands with derivatization reagents. TLC and HPTLC chromatograms often provide semipermanent records of the separation achieved. Further analytical methods can be applied to determine the nature of the sample [25]. Different kinds of chiral selectors were tried for the enantiomer separation of the investigated drugs. These are cyclodextrin, amino acids, macrocyclic antibiotic, and ligand exchange complexes. The best results were obtained by using HP-β-CD – being multimodal selectors since multiple chiral interactions are possible by very different mechanisms. As HP-β-CD molecules are chiral themselves, they can form a diastereomeric pair of inclusion complexes with each enantiomer of a racemate [26]. In HP-β-CD, some hydroxyl groups are substituted with hydroxypropyl functional groups. This modification allows for a more stereospecific and stronger interaction between the hydroxyl groups and hydrogen-bonding moiety present in the drug structure [27]. Cyclodextrins are well suited to chromatography since the inclusion process is stereoselective and reversible. Also, they are stable within a wide range of pH. Other advantages of CDs are UV transparency within the wavelength range commonly used for chromatographic detections, nontoxicity, and resistance to light.

3.1 Optimization of the Enantiomeric Separation

3.1.1 Effects of HP-β-CD Concentration on Enantioseparation

The influence of HP-β-CD concentration was investigated in the range of 0.3–0.7% for zopiclone and 0.1–0.5% for ofloxacin. Chiral resolution was achieved for the concentration ranges of 0.5% and 0.3% for zopiclone and ofloxacin, respectively. The increase of HP-β-CD concentrations and a progressive decrease in enantioseparation were observed, as summarized in **Table 1.** As the concentration of HP-β-CD was increased from 0.3 to 0.5% and 0.1 to 0.3 for zopiclone and ofloxacin, respectively, the resolution tendency becomes flat due to enhanced spot broadening while that of migration time increases significantly. HP-β-CD concentration of 0.5% and 0.3% for zopiclone and ofloxacin, respectively, was adopted as a compromise between resolution and R_F (**Figures 2a and b**).

3.1.2 The Effect of pH on Enantioseparation

The pH value is an important factor for consideration in the separation of enantiomers as it impacts the states of both the solute and the cyclodextrin. Each of zopiclone enantiomers has several nitrogen atoms, amide linkage, while each of ofloxacin enantiomers has several nitrogen atoms and its aliphatic structure. Dissociation equilibrium exists in aqueous solutions: $HA^+ \leftrightarrow A + H^+$.

The dissociation constant can be described by: Ka = [A] [H] / [HA], where [HA^+] and [A] are neutral molecule and ions of (±)-zopiclone or (±)-ofloxacin, respectively. In aqueous phase, both drugs exist in two states of neutral molecules and ionic forms. The study shows that the separation factors were decreased obviously with the increase of pH as the amount of ionic form was increased and the amount of molecular was decreased. The possible reasons for these may be due to the fact that HP-β-CD mainly has chiral recognition ability for zopiclone

Table 4b

Accuracy of the proposed TLC–densitometric method for the determination of levofloxacin in drug substance and drug products.

Conc. (µg spot^{-1})	Drug substance (pure isomer)			Pharmaceutical formulations											
				Oflicin eye drops			Otoxin ear drops			Venaxan vial (I.V. infusion)			Levoflox vial (I.V. infusion)		
	Found conc. (µg spot^{-1})	Recovery[a)] (%)	% RSD	Found conc. (µg spot^{-1})	Recovery[a)] (%) of claimed amount	% RSD	Found conc. (µg spot^{-1})	Recovery[a)] (%) of claimed amount	% RSD	Found conc. (µg spot^{-1})	Recovery[a)] (%) of claimed amount	% RSD	Found conc. (µg spot^{-1})	Recovery[a)] (%) of claimed amount	% RSD
2	2.03	101.50	1.111	1.865	46.62	1.549	1.893	47.32	2.004	1.966	98.30	0.584	1.934	96.70	1.212
3	3.05	101.66	0.985	2.878	47.96	2.008	2.881	48.01	0.877	2.954	98.46	0.611	2.899	96.63	1.044
5	5.11	102.20	1.628	3.844	48.05	1.762	3.862	48.27	1.593	3.882	97.05	1.081	3.797	94.92	0.812
6	6.13	102.16	0.937	5.799	48.32	0.964	5.814	48.45	1.438	5.911	98.51	1.167	5.867	97.93	0.899
7	7.10	101.42	1.891	6.745	48.17	1.549	6.809	48.63	2.004	6.825	97.45	0.743	6.790	97.00	0.557
Mean ± % RSD		101.79 ± 0.360			47.82 ± 1.434			48.14 ± 1.058			97.95 ± 0.676			96.63 ± 1.128	

[a)]Average of four different determinations

or ofloxacin molecules in ionic forms. Therefore, the optimal enantioresolution was obtained at pH 4 and 4.5 for zopiclone and ofloxacin, respectively.

3.1.3 The Effect of Temperatures on Enantioseparation

The effect of temperature within a reasonable range (5–25°C) was investigated. Table 1 shows that a higher temperature leads to a decrease in both distribution ratios and enantioselectivities. A decrease in separation factors can be explained by the fact that the interaction between enantiomer and chiral selector is decreased with the rise of temperature and the discrimination ability of the selectors for zopiclone and ofloxacin enantiomers is decreased as well.

3.2 Thermodynamics of the Chromatographic Separation

The retention behavior and thermodynamic parameters determined in this study were used to estimate the enthalpy, entropy, and Gibbs free energy of association between the two enantiomers and the normal TLC plate stationary phase. Data obtained from retention and separation at temperatures ranging from 5 to 25°C were proposed using the Van't Hoff equation [28]. **Figure 3** shows the variations of ln k and ln α *versus* 1/T. The results can be described as fitting very well with the Van't Hoff model, indicating that the complexes do not change in conformation and the enantioselective interactions remain unchanged in the studied temperature range (Table 1).

The retention factor k can be calculated by Eq. (1):

$$\ln k = -\frac{\Delta H^\circ}{R}\frac{1}{T} + \frac{\Delta S^\circ}{R}, \quad (1),$$

where R and T are the gas constant and absolute temperature, respectively. The enthalpy ($\Delta H°$), entropy ($\Delta S°$), enthalpy difference ($\Delta\Delta H°$), and entropy difference ($\Delta\Delta S°$) can be calculated from the slopes and intercepts of the linear portion of the above equation. The results are listed in **Table 2.** The absolute values of $\Delta H°$ and $\Delta S°$ of (*R*)-ofloxacin were larger than levofloxacin, indicating that (*R*)-ofloxacin could form more stable ternary complexes and has stronger affinity to the normal TLC stationary phase than levofloxacin and the same for zopiclone. The finding that $|\Delta\Delta H°| > T|\Delta S°|$ indicates that chiral separation on normal TLC plate is an enthalpy-controlled process. In this case, the decrease in temperature led to an increase in the separation factors. This difference can be calculated from the retention differences associated to the selectivity, and mathematically described as Eq. (2) [29–31]:

$$\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ \quad (2)$$

The combination of Eqs. (1) and (2) leads to Eq. (3) as follows:

$$\ln \alpha = -\frac{\Delta\Delta H^\circ}{R}\frac{1}{T} + \frac{\Delta\Delta S^\circ}{R} \quad (3)$$

The plots of ln k and ln α *versus* 1/T (Figures 3a and b) show a linear dependence according to the Van't Hoff equation with a mean square deviation (R^2) of 0.972 and 0.978 for zopiclone enantiomers and 0.973 and 0.979 for ofloxacin enantiomers. The negative values of $\Delta H°$ indicate a favorable adsorption of the chiral drug on the stationary phase. Moreover, negative values of $\Delta S°$ indicate an increase in the order of chromatography (stationary phase–mobile phase–enantiomers) when the enantiomers are transferred from the mobile phase to the stationary

Table 5

Precision data for enantioseparation of zopiclone and ofloxacin.

		Zopiclone				Ofloxacin		
		% RSD						
Concentration (μg^{-1})	Parameters	Instrumental repeatability ($n = 6$)	Method repeatability ($n = 6$)	Inter-day precision ($n = 3$)	Concentration ($\mu g\ spot^{-1}$)	Instrumental repeatability ($n = 6$)	Method repeatability ($n = 6$)	Interday precision ($n = 3$)
1	R_F	0.532	0.344	0.813	2	0.548	0.222	0.721
	Peak area	0.474	1.073	0.851		0.369	1.347	0.943
2	R_F	0.308	0.488	0.547	3	0.247	0.494	0.347
	Peak area	0.873	1.275	0.972		0.897	1.008	0.806
3	R_F	0.699	0.673	1.073	5	0.612	0.372	1.463
	Peak area	0.307	0.907	0.855		0.347	1.506	1.479
4	R_F	1.111	0.533	0.647	7	1.287	0.473	0.652
	Peak area	1.493	1.003	1.123		1.477	1.233	1.087
% RSD	R_F	0.512	0.266	0.298	% RSD	0.651	0.318	0.595
	Peak areas	0.670	0.146	0.134		0.691	0.164	0.269

phase. All of the parameters $\Delta H°$, $\Delta S°$, $\Delta\Delta H°$, and $\Delta\Delta S°$ are clearly negatives meaning that the separation is enthalpy-controlled.

3.3 Validation Results

Good linearity was observed for pure isomers: eszopiclone and levofloxacin, respectively **(Figures 4a and b),** with typical linear regression equations as presented in **Table 3.** LOD and LOQ were calculated according to ICH guidelines, and the result is shown in Table 3. The result of method precision expressed in terms of relative standard deviation (% RSD) values for R_F and peak areas for the active isomers is presented in Table 3. Accuracy was determined with quality-control samples. Good recoveries were obtained for the active and racemic forms as given in **Tables 4a and b.** The result of method precision expressed in terms of % RSD values for R_F and peak areas for the active isomers is shown in Table 3. These results indicate a good precision of the developed methods **(Table 5).**

4 Conclusion

The proposed method exhibited superior performance in terms of sensitivity, dynamic concentration range, and selectivity. Stereospecificity was achieved by using HP-β-CD as chiral mobile phase additive. The effects of different separation conditions were investigated, and the thermodynamics data revealed that enantioseparation is an enthalpy-controlled process. The proposed method was successfully used for the quality evaluation of chiral impurities of eszopiclone and levofloxacin in their drug substances and drug products. This expedient greatly simplified the overall procedure, resulting in a rapid and efficient sample analysis while maintaining precision and accuracy.

References

[1] *S.C. Sweetman,* Martindale – The Complete Drug Reference, 36th edn., The Pharmaceutical Press, London, 2009.

[2] *C.J. Halas,* Am. J. Health Syst. Pharm. **63** (2006) 41–48.

[3] *K. Tang, Y. Chen, J. Liu,* Sep. Purif. Technol. **62** (2008) 681–686.

[4] *S. Piperaki, M. Parisi-Poulo,* J. Chromatogr. A **729** (1996) 19–28.

[5] *C. Fernandez, B. Baune, F. Gimenez, A. Thuillier, R. Farinotti,* J. Chromatogr. **572** (1991) 195–202.

[6] *R.T. Foster, G. Caillé, A.H. Ngoc, C.H. Lemko, R. Kherani, F.M. Pasutto,* J. Chromatogr. B **658** (1994) 161–166.

[7] *C. Fernandez, B. Baune, F. Gimenez, A. Thuillier, R. Farinotti,* J. Chromatogr. **617** (1993) 271–280.

[8] *F.T. Misuhashi,* Chemotherapy **36** (1990) 268–276.

[9] *I. Hayakawa, S. Atarashi, S. Yokohama, M. Imamura, K. Sakano, M. Furukawa,* Antimicrob. Agents Chemother. **29** (1986) 163–164.

[10] *K.H. Lehr, P. Damm,* J. Chromatogr. **425** (1988) 153–161.

[11] *T. Arai, H. Koike, K. Hirata, H. Oizumi,* J. Chromatogr. **448** (1988) 439–444.

[12] *B. Koppenhoefer, U. Epperlein, R. Schlunk, X. Zhu, B. Lin, B,* J. Chromatogr. A **793** (1998) 153–164.

[13] *S. Zhou, J. Ouyang, W.R.G. Baeyyens, H. Zhao, Y. Yang,* J. Chromatogr. A **1130** (2006) 296–301.

[14] *T.D. Boer, R. Mol, R.A.D. Zeeuw, G.I.D. Jong, K. Ensing,* Electrophoresis **22** (2001) 1413–1418.

[15] *T. Arai, N. Nimura, T. Kinoshita,* J. Chromatogr. A **736** (1996) 303–311.

[16] *X.F. Zhu, Y.S. Ding, B.C. Lin, A. Jakob, B. Koppenhoefer,* Electrophoresis **20** (1999) 1869–1877.

[17] *T. Arai, M. Ichinose, H. Kuroda, N. Nimura, T. Kinoshita,* Anal. Biochem. **217** (1994) 7–11.

[18] *T. Horimai, M. Ohara, M. Ichinose,* J. Chromatogr. A **760** (1997) 235–244.

[19] *N.M. Maier, P. Franco, W. Linder,* J. Chromatogr. A **906** (2001) 3–33.

[20] The British Pharmacopoeia, Vol. 4, Her Majesty's Stationery Office, London, 2013.

[21] ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures, Text and Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva, 2005.

[22] *E.J. Ariens, W. Soudijn, P.B.M.W.M. Timmermans,* Stereochemistry and Biological Activity of Drugs, Blackwell Scientific Publications, Oxford, 1983.

[23] *M. Eichelbaum, A.S. Gross,* Adv. Drug Res. **28** (1996) 1–64.

[24] *M. Mack, H.E. Hauck,* Chromatographia **26** (1988) 197–205.

[25] *D. Stevenson, I.D. Wilson,* Recent Advances in Chiral Separations, Plenum Press, New York, 1991.

[26] *T.E. Beesley, R.P.W. Scott,* Chiral Chromatography, John Wiley & Sons Ltd., New York, 1998.

[27] *A.M. Stalcup, S. Chin Chang, D.W. Armstrong, J. Pitha,* J. Chromatogr. **513** (1990) 181–194.

[28] *J. Chmielowiec, H. Sawitzky,* J. Chromatogr. Sci. **17** (1979) 245–252,

[29] *M. Schlauch, A.W. Frahm,* Anal. Chem. **73** (2001) 262–266.

[30] *T. Rojkovičová, J. Lehotay, J. Krupčík, A. Fedurcová, J. Čižmárik, D.W. Armstrong,* J. Liq. Chromatogr. Rel. Technol. **27** (2004) 1653–1671,

[31] *H. He, Y. Liu, C. Sun, X. Wang, C.P. Huy,* J. Chromatogr. Sci. **42** (2004) 62–66.

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