Research paper

Development and Validation of Densitometry-TLC Stability Indicating Methods for Quantitative Determination of Azelastine Hydrochloride and Emedastine Difumarate in their Drug Products

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Abstract

Stability indicating densitometry-TLC assay was established and validated for determination of azelastine hydrochloride and emedastine difumarate in the presence of their acid and oxidative degradants. Forced degradation was performed using 30% H_2O_2 and 5 M HCl. The method was based on thin-layer chromatographic separation of the two drugs from their degradants, using methanol-ammonia (9.5:0.5, *v/v*) as developing system, followed by densitometric measurements of the intact drug spots at 292 and 283 nm, respectively. The linear range was 0.5 - 10.0 µg/spot, with mean recoveries of 100.09 ± 0.53% and 100.36 ± 0.40%. The proposed method was successfully applied for the routine quality control analysis of the two drugs in synthetic mixtures and commercially available preparations. The degradation products were identified by IR and MS and the pathways were illustrated. The method was validated according to ICH.

Keywords: Azelastine hydrochloride, Emedastine difumarate, Densitometry-TLC, Stability, Validation

Introduction

Azelastine-HCl(AZT) is 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl] phthalazin-1(2H)-one hydrochloride[1]. It is an intranasal antihistamine indicated for use in patients with seasonal allergic rhinitis and non-allergic vasomotor rhinitis**.** It is also used topically in the symptomatic relief of allergic conditions including rhinitis and conjunctivitis[2,3]. Emedastine difumarate(ETD) is 1H-benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1H-1, 4- diazepin-1-yl), (E)-2-butenedioate(1:2)[4]. It is a second generation antihistamine used in eye drops to treat allergic conjunctivitis[5].

The available methods for analysis of azelastine-HCl in pharmaceutical dosage forms and biological fluids are volumetric[6], UV spectrophotometry[7], colorimetry[7], TLC[8], HPLC[9-11], and capillary electrophoresis[12]. Few methods were reported for analysis of emedastine difumarate include only HPLC with tandem MS[13,14] or radioreceptor assay[15]detectors. The international Conference on Harmonization (ICH) guidelines recommended stress testing to elucidate the inherent stability of active substances[16].

In the literature, no method is available so far for separation and structure elucidation of the hydrolytic and oxidative degradants of AZT and ETD. Therefore, the aim of the present work is to establish densitometry-TLC method for the selective determination of both drugs.

Experimental

Instrumentation

- Shimadzu dual wavelength flying spot densitometer Model CS - 9301 PC (Tokyo – Japan).
- Hamilton micro syringe (25µL).
- Aluminum plates (20 cm x 20 cm), coated with 0.2 mm layers of nano-silica gel 60 with fluorescence indicator, (Macherey – Nagel, Germany).
- UV short wavelength (254 nm) Lamp, (Desaga, Germany).
- A Bruker Vector 22 spectrometer (Bruker Instruments Ltd, Rheinstetten/ Karlsruhe, Germany) was used for recording IR spectra using KBr pellets in the range (4000 - 400 cm^{-1}).
- A Shimadzu GCMS-QP1000 EX quadrupole spectrometer. EI ionization was performed with an electron energy of 70 eV. The ion source temperature was 200 ^{o}C, scan mode was ACQ, and scan speed was 769 U s^{-1}.

Materials and Reagents

Azelastine-HCl was kindly supplied from European Egyptian Pharm Co., Egypt, with certified purity of 99.00%. Zalastine® Nasal Spray labeled to contain 1 mg azelastine-HCl per mL (BN 7579001, European Pharm Co., Egypt) and Azelast® Eye Drops, labeled to contain 0.5 mg azelastine-HCl per mL (BN 86872, product of El-Kahira Pharm and Chem Ind Co., EPCI, Egypt) were purchased from the local market. Emedastine difumarate was kindly supplied from Chem Swiss, SIGMA Co., Egypt with purity 99.00% [4]. Emedastine 0.05% Ophthalmic Solution® labeled to contain 0.5 mg Emedastine difumarate per 1 mL (Batch no., 190409-F_1, manufactured by SIGMA Co. Egypt) was purchased from the local market. Hydrochloric acid(Fischer Scientific, UK), ammonia(10% aqueous, Adwic Co., Egypt), hydrogen peroxide(30%, Adwic Co., Egypt) and NaOH(Adwic Co., Egypt) an methanol(Lab. Scan, Ireland) were used.

Standard Solutions

Standard stock solutions of AZE and ETD (1 mg mL) were prepared in methanol and diluted with methanol to obtain working solutions of 5 - 100 μg mL^{-1} for each drug. The stock solutions were stable for one week at 4 ^oC.

Preparation of Degradation Products

Acid degradants

About 50 mg of azelastine-HCl or emedastine difumarate were refluxed with 50 mL 5 M HCl at 100 ^oC for 36 hours or 7 hours respectively, then neutralized with 5 M NaOH and evaporated to dryness under vacuum. The residue of each drug was extracted with 3x10 mL methanol, filtered into 50 mL volumetric flasks and the volume was completed with methanol. The obtained solutions were labeled to contain the acid degradants derived from 1 mg mL^{-1} of each drug.

Oxidative degradants

About 50 mg of each drug were weighed in 50 mL volumetric flask, completed to the mark with 30% H_2O_2, and left in the dark for 24 hours for azelastine-HCl and 6 hours for emedastine difumarate. Both solutions were evaporated to dryness under vacuum. The residues were dissolved in 40 mL methanol and transferred separately to 50 mL volumetric flasks. The volume was completed with methanol to obtain a solution labeled to contain the oxidative degradants derived from 1 mg mL^{-1} of each drug.

Densitometry-TLC Method

TLC was performed on 20 x 20 cm aluminum plates precoated with silica gel F254, 10 µL of each azelastine-HCl or emedastine difumarate were applied to the plates with 25 µL Hamilton microsyringe. Ascending development of the plates, with methanol-10% ammonia(9.5:0.5, *v/v*) as mobile phase, was performed. After development, the plates were air-dried and scanned at 292 nm and 283 nm for AZT and ETD respectively in reflection photo mode and zigzag scan, with swing width=10.

Laboratory Prepared Mixtures

Aliquots of each standard drug solution (1 mg mL^{-1}) equivalent to 90 - 10 µg were transferred into a series of 10 mL volumetric flasks. Then mixed with volumes of the corresponding drug – acid or oxidative degradants (*prepared as mentioned under 2.4*) derived from 10 – 90 µg azelastine-HCl or emedastine difumarate. Volumes were completed with methanol and the detailed under, "*2.5 Densitometry-TLC Method*" were followed. Intact drug concentrations were calculated from the corresponding regression equation.

Application to Pharmaceutical Formulations

The content of five bottles of Zalastine nasal spray or twelve bottles of Azelast eye drops were mixed and a volume equivalent to 25 mg azelastine-HCl was evaporated under vacuum. The residue was extracted with 2 x 10 mL methanol and filtered into 25 mL volumetric flask, and completed with methanol.

The contents of eighteen Emedastine 0.05% ophthalmic bottles were mixed and volume equivalent to 25 mg emedastine base was evaporated under vacuum and above details were followed. The obtained methanolic solutions labelled to contain 1 mg mL^{-1} of the each drug were analyzed by the proposed densitometric-TLC method as described under " *Densitometry-TLC Method* ". The concentration of each drug was calculated from the corresponding regression equation.

Results and Discussion

Forced degradation of both azelastine-HCl and emedastine difumarate has been studied through acid and oxidative stress conditions. Partial hydrolysis (about 50% as measured by the proposed densitometry TLC method) of azelastine-HCl was achieved after reflux with 5 M HCl for 36 hours or about 65% of emedastine difumarate after reflux with 5 M HCl for 7 hours. Also partial oxidative degradation (about 50%) of azelastine-HCl was obtained by keeping 1 mg mL^{-1} solution of it in 30% H_2O_2 for 24 hours. While complete oxidative degradation of emedastine difumarate was obtained by keeping 1 mg mL^{-1} solution in 30% H_2O_2 for 6 hours.

Separation and identification of degradants

The methanolic extracts of acid-hydrolysis and oxidative degradation products of each drug was tested by TLC on silica gel 60 F254 plates. Different developing systems were tried as mixture of toluene –methanol – chloroform - 10% ammonia, and mixture of dichloromethane – methanol - triethylamine in different ratios no separation was achieved. Complete resolution of each drug from its degradants was achieved upon using a mixture of methanol – 10% ammonia (9.5: 0.5 *v/v*) with detection under UV lamp at 254 nm.

For acid hydrolyzed azelastine-HCl, two spots with R_f 0.66 and R_f 0.84 were appeared and two spots at R_f 0.73 and R_f 0.86 for its oxidative degradation products. Whereas the intact drug spot was at R_f 0.59, Figure (1). However, for emedastine difumarate one spot at R_f 0.73 for acid hydrolyzed solution and one spot at R_f 0.4 for its oxidative degradant. Intact emedastine base spot appeared at R_f 0.6 and difumarate spot appeared at R_f 0.79 as shown in Figure (1).

The degradants of each drug was subsequently separated on preparative TLC plates using the same developing solvents and extracted with methanol. The methanolic solutions were evaporated under vacuum, the residues were confirmed by IR on KBr discs and mass spectroscopy, and the results are given in Table 1. The suggested pathway of azelastine HCl and emedastine difumarate degradation are shown in Scheme (1&2).

Method Validation[17]

Linearity range

Good correlation was found to exist between the peak areas of the separated spots and drug concentration over the range of 0.5 - 10 µg/spot for azelastine-HCl and emedastine difumarate as indicated by correlation coefficient (r= 0.9993 - 0.9997), Table 2.

The limit of detection (LOD)

The LOD was calculated to be 0.031 and 0.042µg/spot for azelastine-HCl and emedastine base, respectively, Table 2.

Accuracy

The previously mentioned procedure under linearity was repeated three times for five different concentrations within the linearity range. The mean percentage recoveries were ranged between 100.09% and 100.36% for the two drugs respectively, Table 2.

Precision

The precision of the assay (within assay and between assays) was determined for both drugs in triplicate at five concentration levels for each drug using the previous mentioned procedure under linearity in the same day Table 2. The intra day, RSD were 0.641-1.27% and 0.367-1.09% while intermediate RSD were 0.891–1.42% and 0.438 – 1.31% for AZT and EDT respectively, Table 2.

Selectivity

The selectivity of the proposed method was revealed by analyzing laboratory prepared mixtures of intact drugs with each of its degradants. The method was applicable for the selective determination of intact AZT in presence of, 10 – 45 % of its acid or oxidative degradants(Table 3) with mean recoveries of 99.42 ±1.59 % and 100.2 ± 1.44 % respectively. Table (4) shows that EDT could be determined in presence of up to 59 % of its acid degradant or up to 80 % of oxidative degradant with mean recoveries of 99.34 ± 1.55 and 99.58 ± 1.16%, respectively.

Robustness

Robustness was assessed by evaluating the influence of small variation of experimental variables as developing system composition, saturation time and temperature on reliability of the method. For mobile phase, methanol: ammonia 10%, 9.5:0.4 or 9.5:0.3 gave RSD% not exceeding 1.52% for azelastine-HCl and 1.44% for emedastine. The small change in temperature 23, 25, 27 ^{o}C or saturation time (15±2 min) did not significantly affect the results.

Application of the proposed densitometric-TLC method

The proposed method was applied for the determination of the two drugs in their pharmaceutical preparations. The results revealed good recoveries ± RSD% of 99.33 ±1.210 %, 99.25 ± 1.176% for azelastine-HCl in Zalastine nasal spray or Azelast eye drops and 99.37 ±1.410 % for emedastine base in Emedastine ophthalmic solution.

Statistical analysis of the results obtained by the suggested method compared with the manufacturer[18] or official method for azelastine-HCl[4] and emedastine difumarate[1] revealed no significant difference within a probability of 95%; Table (5). However, the proposed densitometric-TLC method is more sensitive and more selective than the manufacturer or reported methods in being stability indicating one.

The validity of the proposed method was further assured by applying the standard addition technique. The mean percentage recoveries ± RSD% were 99.74 ± 1.381 and 99.56 ± 1.121 for azelastine-HCl and 99.95 ±1.412 for emedastine base; Table (6).

Conclusion

The suggested densitometric-TLC method proved to be pure green analytical chemistry, as the solvent used have minimal hazards. The method can be used for routine analysis of azelastine hydrochloride and emedastine in their drug substances, drug products and in presence of their acid and oxidative degradants. The method complied with the validation guidelines of the International Conference on Harmonization and could be used for purity testing, stability studies, and quality control of both drugs.

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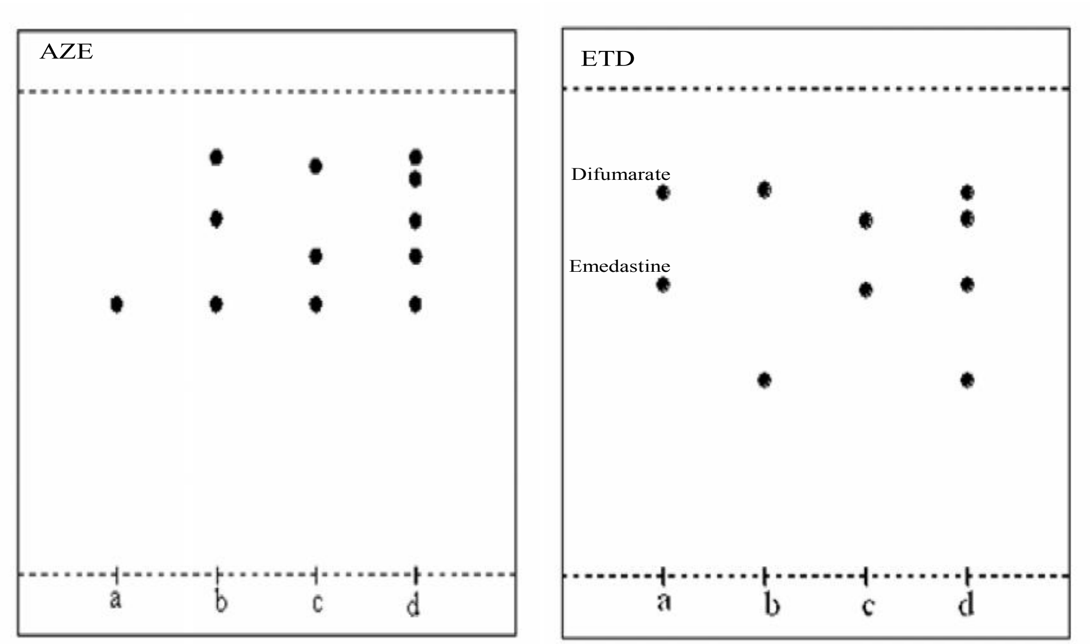


Fig. 1 Thin layer chromatogram of (a) azelastine-HCl or emedastine and their(b) oxidative degradants (c) acid degradants (d) mixture of pure and degraded drugs, developing system: methanol-10% aqueous ammonia (9.5: 0.5 *v/v*).

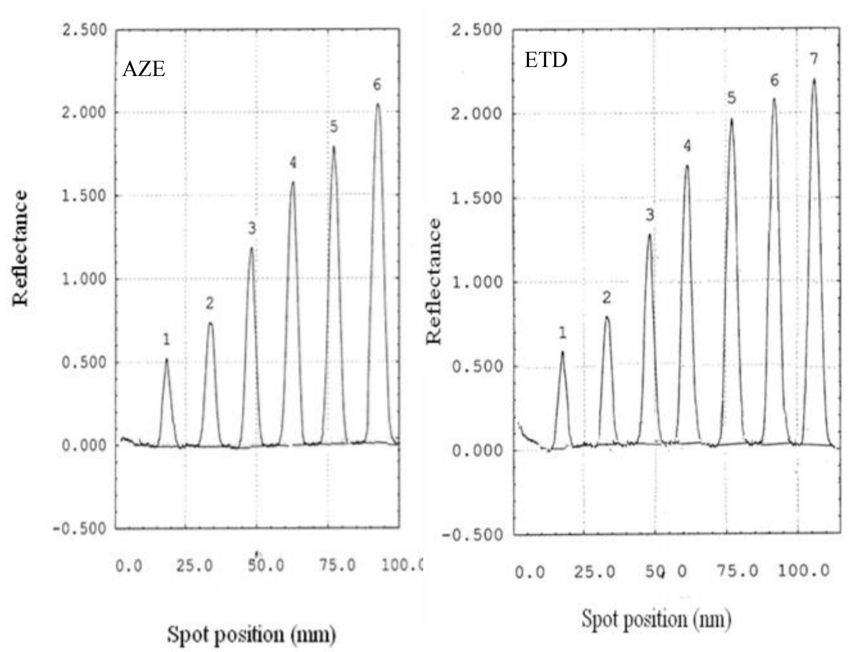
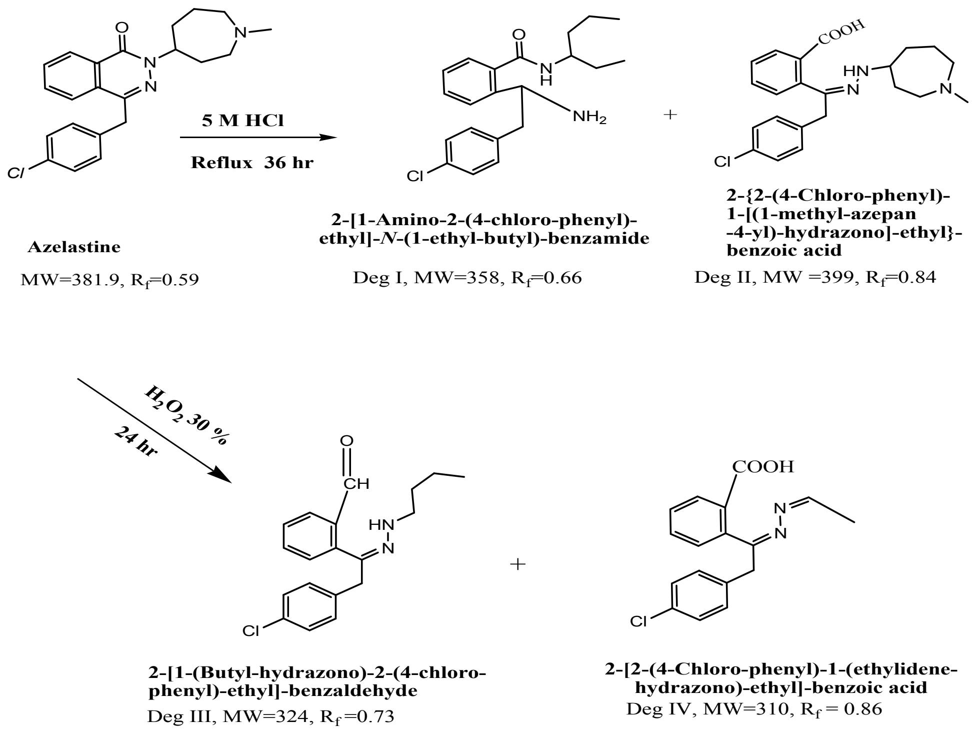
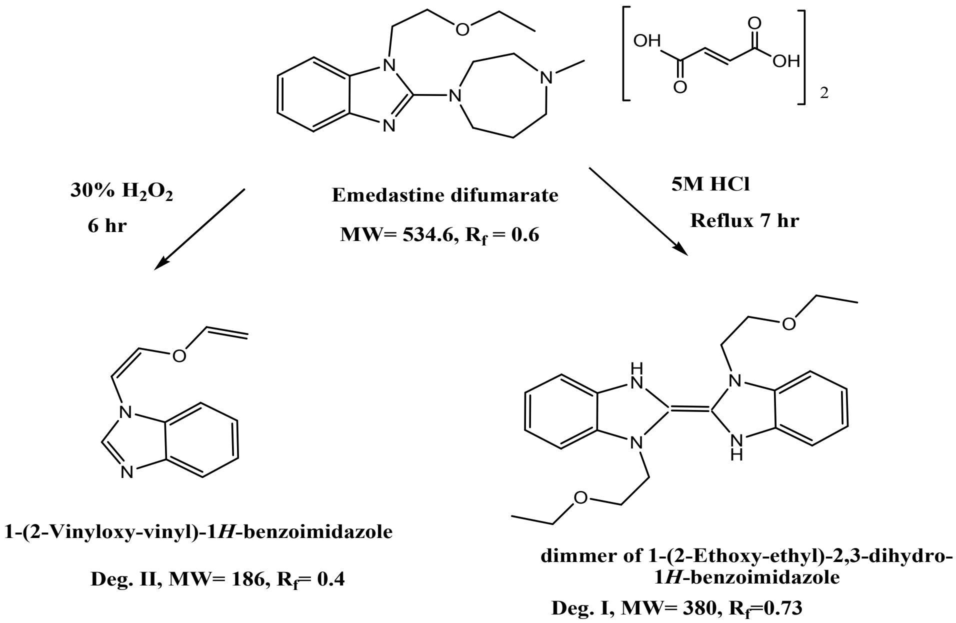


Fig. 2 Densitometric scanning profile for TLC-chromatogram of different concentrations (0.5 - 10 µg/spot) at 290 and 283 nm for azelastine-HCl and emedastine respectively.



Scheme 1. Suggested pathway for the acid and oxidative degradation of azelastine-HCl.



Scheme 2. Suggested pathway for acid and oxidative degradation of emedastine difumarate.

Table 1. Assignment of IR and mass data of azelastine-HCl and emedastine base and their degradants

Drug	Mass data	IR data
Azelastine-HCl	m/z=381.3 (M) corresponding to $C_{22}H_{24}ClN_3O$	Band at 1330 cm^{-1} (ν C-N) of seven ring
Acid degradate (I)	m/z=356 (M-2) corresponding to $C_{21}H_{24}ClN_2O$	Band at 1628 cm^{-1} (ν C=O) of CO-NH. Band at 3417 cm^{-1} of NH_2
Acid degradate (II)	m/z =397 (M-2) corresponding to $C_{22}H_{24}ClN_3O_2$	Band at 1734 cm^{-1} (ν C=O) of COOH. Band at 3433 cm^{-1} (ν O-H) of COOH
Oxidative degradate (III)	m/z =323 (M - 1) corresponding to $C_{19}H_{18}ClN_2O$	Band at 1731 cm^{-1} (ν C=O) of CHO.
Oxidative degradate (IV)	m/z =308 (M - 2) corresponding to $C_{18}H_{17}ClN_2O$	Band at 1732 cm^{-1} (ν C=O) of COOH.
Emedastine difumarate	m/z=534.6 (M) corresponding to $C_{25}H_{34}N_4O_9$	Band at 1719 cm^{-1} (ν C=O) of COOH. Band at 1016.3 cm^{-1} (ν –O-) of aliphatic ether
Acid degradate (I)	m/z=380 (M) corresponding to $C_{22}H_{28}N_4O_2$	Band at 1654 cm^{-1}, ν double bond of dimmer
Oxidative degradate (II)	m/z= 185(M-1) corresponding to $C_{11}H_{10}N_2O$	Band at 1103 cm^{-1} (ν –O-) of aromatic ether

Table 2. Assay validation parameters of the proposed densitometric-TLC method

Parameters	Azelastine-HCl	Emedastine difumarate
Linearity (µg/spot)	0.5 - 10.0	0.5 - 10.0
LOD (µg/spot)	0.031	0.042
Accuracy(mean±RSD)		
Intraday	98.0 - 100.88	99.58 - 100.41
Interday	98.63 - 101.60	98.50 - 101.02
Precision(RSD%)		
Intraday	0.641 - 1.27	0.367 - 1.09
Interday	0.891 - 1.420	0.438 - 1.31
Regression parameters		
Slope	9.77 x10^2	9.36 x10^2
SD of the slope	1 x10^{-2}	0.81 x10^{-2}
Intercept	2.99 x10^2	10.45 x 10^2
SD of the intercept	0.9 x10^{-2}	0.78 x10^{-2}
Correlation coefficient (r)	0.9993	0.9997

Table 3. Determination of azelastine-HCl in mixtures with its acid or oxidative degradation products by the proposed densitometric- TLC method

Intact* (µg/spot)	Acid degradants (µg/spot)	Recovery % of intact*	Oxidative degradants (µg/spot)	Recovery% of intact[a]
5.5	4.5	98.00	4.5	101.32
6	4	98.21	4	98.73
7	3	101.10	3	98.55
8	2	98.60	2	101.50
9	1	101.20	1	100.90
Mean ± RSD%		99.42 ±1.59		100.2 ± 1.44

[a]Added + remained in degraded solution.

Table 4. Determination of emedastine base in mixtures with its acid or oxidative degradation products by the proposed densitometric-TLC method

Intact* (µg/spot)	**Acid degradants (µg/spot)**	**Recovery% of intact[a]**	**Intact (µg/spot)**	**Oxidative degradants (µg/spot)**	**Recovery % of intact**
4.15	5.85	98.08	1	9	98.90
4.80	5.20	101.03	2	8	100.50
6.10	3.90	98.03	4	6	101.10
7.40	2.60	101.02	6	4	99.00
8.70	1.30	98.55	8	2	98.40
Mean ± RSD%		99.34 ± 1.55			99.58 ± 1.16

[a]Added + remained in degraded solution.

Table 5. Statistical analysis of the results obtained by the proposed densitometric-TLC and manufacturer or official methods for the determination of azelastine-HCl and emedastine base

Parameters	Drug substances				Drug products					
	Azelastine-HCl		Emedastine base		Zalastine nasal spray		Azalast eye drop		Emedastine ophthalmic solution	
	Proposed TLC method	Official[a] method	Proposed TLC method	Official[b] method	Proposed TLC method	Manufac.[c] method	Proposed TLC method	Manufac.[c] method	Proposed TLC method	Offical[b] method
Mean %	100.09	100.3	100.36	100.3	99.33	99.52	99.25	99.26	99.37	100.6
SD	0.528	0.543	0.395	0.819	1.21	1.02	1.176	1.11	1.410	0.938
Variance	0.279	0.295	0.156	0.671	1.464	1.04	1.383	1.23	1.988	0.879
n	5	5	5	5	5	5	5	5	5	5
t-test (2.306)[d]	0.620		0.147		0.201		0.014		1.625	
F-test (6.400)[d]	1.06		4.3		1.41		1.12		2.26	

[a]Official HPLC method (BP 2012). [b]Official HPLC method (USP 2012). [c]Manufacturer's UV spectrophotometric method.

[d]The values between paranthesis are the theoretical values of t and F at (p=0.05).

Table 6. Application of standard addition technique for the determination of azelastine HCl and emedastine base by the proposed densitometric-TLC method

Conc.(µg/spot)		Zalastine nasal spray	Azalast eye drop	Emedastine ophthalmic soln.
Claimed taken	**Pure added**	**Recovery[a]% of pure added**	**Recovery[a]% of pure added**	**Recovery[a]% of pure added**
2	1	98.63	98.44	99.32
2	2	98.84	101.11	101.21
2	4	101.55	100.34	98.97
2	6	100.91	98.82	98.56
2	8	98.77	99.10	101.71
Mean recovery ±RSD%		99.74 ± 1.381	99.56 ± 1.121	99.95 ± 1.412

[a]Average of three determinations.