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Stability indicating spectrophotometric and chromatographic methods for the determination of azelastine hydrochloride in presence of its alkaline degradant

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ABSTRACT

Five simple, rapid and sensitive methods have been developed for the determination of azelastine hydrochloride in presence of its alkaline degradant. The first method utilizes first (D_1) derivative spectrophotometry at 260 and 321 nm for the determination of azelastine hydrochloride without interference from its degradation product. The second method depends on the use of the first derivative of the ratio spectra (DD_1) with measurement at 321 nm for determination of azelastine hydrochloride using the spectrum of 20 μg ml^{-1} (degradation) as a divisor. The third method is mean centering of ratio spectra with measurement at 306.4 nm.The fourth method depends on TLC-densitometry using acetonitrile: water: acetic acid (8:2:1, by volume) as a developing solvent, followed by measurement at 254 nm. The last method is a reversed-phase liquid chromatography using 5mM ammonium acetate (pH 3.2): acetonitrile (40:60, v/v) as the mobile phase with UV detection at 253 nm. The suggested procedures were checked using laboratory prepared mixtures and were successfully applied for the analysis of its pharmaceutical preparations. The validity of the proposed methods was further assessed by applying the standard addition technique. The results obtained by applying the proposed methods were statistically analyzed and compared with the manufacturer's method.
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KEYWORDS

Azelastine hydrochloride;
Derivative;
Derivative ratio;
MCN;
TLC;
RP-HPLC.

INTRODUCTION

Azelastine-HCl (AZT) is 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl] phthalazin-1(2H)-one hydrochloride[1].

This medicament is used topically in the symptomatic relief of allergic conditions, including rhinitis and conjunctivitis[2].

The methods available for analysis of azelastine-HCl in pharmaceutical dosage forms and biological fluids include UV- spectrophotometric methods[3], colorimetric methods[4], potentiometric method[5], capillary electrophoresis method[6], high performance thin layer chromatography (HP-TLC) method[7] and high performance liquid chromatography (HPLC) methods[8-15].

The purpose of this study was to determine azelastine-HCl in presence of its alkaline degradant by simple, accurate, rapid, sensitive and selective spec-

trophotometric and chromatographic methods that can be used for quality control and routine analysis.

EXPERIMENTAL

Instruments

- Spectrophotometer: all absorption spectra and derivatives were recorded with an ultraviolet- (UV) 1650 PC UV-visible double beam spectrophotometer with 1 cm quartz cuvetts, (Shimadzu Corporation, Kyoto, Japan).
- The HPLC was performed on a Shimadzu instrument, Model 10 A VP., equipped with a variable-wavelength detector and a 20-µl volume injection loop.(waters, Milford, USA) XTerra™ RP C_{18} (5 µm, 25 cm x 4.6 mm I.D.) column was used as stationary phase.
- Camag Linomat 5 autosampler with camag microsyringe (100 µL); CAMAG, Muttenz, Switzerland.
- Camag TLC scanner 3 densitometer model 3 S/N 130319 equipped with wincats software for densitometric evaluation; CAMAG, Muttenz, Switzerland.
- Thin-layer chromatographic (TLC) plates; precoated with silica gel 60 F_{254}, 20x20 cm, 0.25 mm thickness (E. Merck, Darmstadt, Germany).
- UV lamp -short wavelength 254 nm, Spectroline®, model CM-10(Westbury, New York, U.S.A.).
- Chromatographic tank 20 x21x 9 cm.

Chemicals and reagents

All chemicals and reagents are of pure analytical grade methanol, glacial acetic acid, ammonium acetate, 2 M hydrochloric acid and 2M sodium hydroxide, all were obtained from (El-Nasr pharmaceutical chemicals, Cairo, Egypt). HPLC-grade acetonitrile (Mumbai, India) and De-ionized water.

Samples

Pure samples

Azelastine-HCl (AZT) was kindly supplied from European Egyptian Pharm Co., Egypt, with 99.26 % purity according to manufacturer's method[16].

Market sample

Azelast eye drops ("BN 16209", El-Kahira Pharm and Chem Ind Co., for EPCI, Cairo Egypt) labeled to contain 0.5 mg AZT per 1 mL.

Degraded sample

Preparation of azelastine hydrochloride alkaline degradant.

It was prepared by dissolving 50 mg of AZT in 50 ml 2 M aqueous NaOH and refluxing for 7 hours. The solution was neutralized using 2 M HCl, evaporated to dryness on hot plate and the residue was dissolved in 20ml methanol. The obtained solution was filtered; the methanol then evaporated to get the azelastine degradant in pure powdered form.

Complete degradation was confirmed by spotting on TLC plates using acetonitrile: water: glacial acetic acid (8: 2: 1, by volume) as a developing system. The spots were dried and visualized under UV light at 254nm. The structure of the degradation product was elucidated by IR and mass spectroscopy.

Stock solutions

Azelastine-HCl (AZT) and its alkaline degradant stock solutions (1.0 mg mL^{-1}), in methanol for D_1, DD_1 and TLC or in mobile phase for HPLC.

Working solutions

100 µg mL^{-1} in methanol for D_1, DD_1 and TLC methods, or in mobile phase for HPLC method.

Methods

Spectrophotometric methods

D_1 spectrophotometric method

Accurate aliquots equivalent to 100-900 µg mL^{-1} of (AZT) were transferred from its working solution (100 µg mL^{-1}) into a set of 10-mL volumetric flasks and diluted to volume with methanol. The absorption was recorded for each solution then the D_1 spectrum was obtained using "λ = 4 and scaling factor = 10. Calibration curve relating the peak amplitudes at 260 and 321nm to the corresponding concentrations was constructed, and the corresponding regression equation was computed.

DD_1 method

The absorption spectra of azelastine HCL in the range of (10-90 µg mL^{-1}) were divided by absorption spectrum of its degradant (20 µg mL^{-1}), and the ob-

tained ratio spectra were differentiated with respect to wavelength. The peak amplitude at 321nm was measured and the calibration curve representing the relation between peak amplitude and the corresponding concentrations was constructed, and the regression equation was derived.

MCN method

The absorption spectra of azelastine HCL in the range of (10-90 μg mL^{-1}) were divided by absorption spectrum of its degradant (20 μg mL^{-1}) and the obtained ratio spectra were mean centered using Matlab software and the concentration of AZT were determined by measuring the amplitude at 306.4 nm.

Chromatographic methods

TLC-Densitometry method

Aliquots of azelastine hydrochloride stock solution (1.0 mg ml^{-1}) were spotted on a TLC plate (20 x 20 cm) with a space of 1 cm between each spot and 2 cm up from the bottom edge of the plate. The chromatographic chamber was equilibrated with the developing solvent, acetonitrile: water: glacial acetic acid (8: 2: 1, by volume), for 30 min. The plate was developed at room temperature by ascending technique to a distance of 18 cm, removed from the chamber and dried. The spots were scanned at 254 nm. Calibration curve relating the peak areas to the corresponding concentrations of AZT was plotted, and the corresponding regression equation was calculated.

HPLC method

Accurate aliquots (0.5-3.0 ml) of (AZT) were transferred from its working solution (100 μg mL^{-1}) into set of 10-mL volumetric flasks and diluted to volume with the mobile phase. Using the Shimadzu instrument, the chromatogram was recorded under the following instrumental parameters: flow rate, 1.5 mL/min at ambient temperature and the eluent monitored at 253 nm. The separation was done on a C18 column using 5 mM ammonium acetate (pH=3.2): acetonitrile (40: 60, v/v) as the mobile phase. Calibration curve for azelastine HCl was plotted, and the regression equation was calculated.

Application to pharmaceutical preparation

Aliquot of 10 mL from dosage form (Azelast), equivalent to 5 mg azelastine hydrochloride, was transferred accurately to a 50-mL volumetric flask and diluted to volume with methanol (D_1 and DD_1) or with by mobile phase in HPLC. For TLC, the aliquots were drawn directly from the dropper. The general procedures under linearity were followed and the concentration of AZT was calculated from the corresponding regression equation.

The validity of the methods was assessed by applying the standard addition technique.

RESULTS AND DISCUSSION

Azelastine hydrochloride is liable to alkaline hydrolysis where complete degradation was obtained after reflux with 2M sodium hydroxide for 7 hrs.

The obtained degradant was separated by TLC on silica gel G F_{254} plates, using acetonitrile: water: glacial acetic acid (8: 2: 1, by volume) as developing solvent.

The structure of the alkaline degradation was elucidated by IR and mass spectroscopy. Figures (1) and (2) show that the IR peak at 1651.07 cm-1 corresponding to the amide in the drug disappeared and new peak at 3425.58 cm-1 appeared corresponding to hydroxyl group (OH) of the acid, as in the suggested scheme of the alkaline degradation shown in the Figure (3). The mass spectrum Figure (4) showed the existence of a peak at m/z 428 corresponding to azelastine alkaline

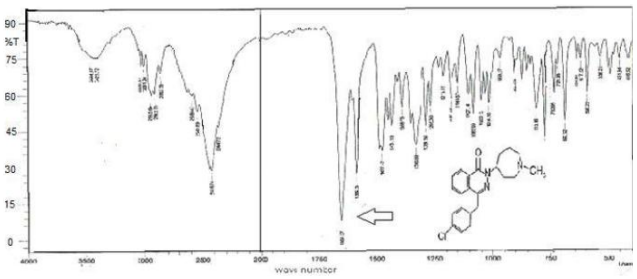

Figure 1 : IR spectrum of azelastine hydrochloride

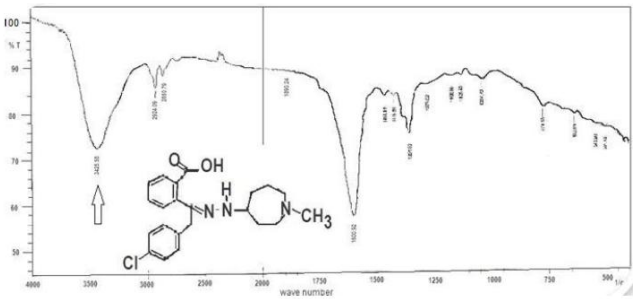

Figure 2 : IR spectrum of azelastine alkaline degradant

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degradation represented in Figure (3). Reviewing the literature in hand shows that there no analytical method is reported for the determination of AZT in presence of its alkaline degradant. Therefore, the aim of this work was to develop and validate stability indicating methods for the determination of AZT in pure form and in pharmaceutical form.

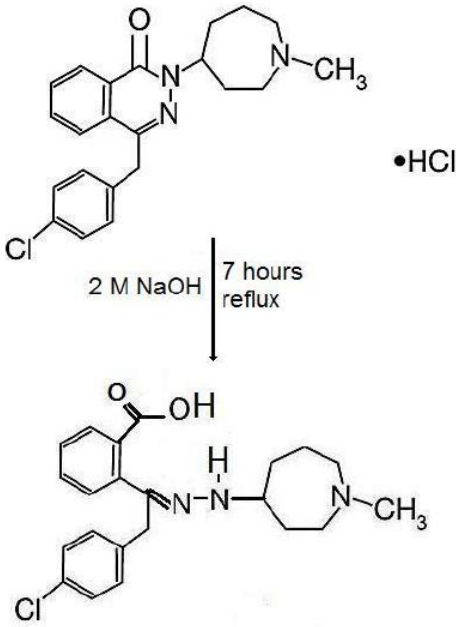

Figure 3 : Suggested scheme for the alkaline degradation of the azelastine hydrochloride

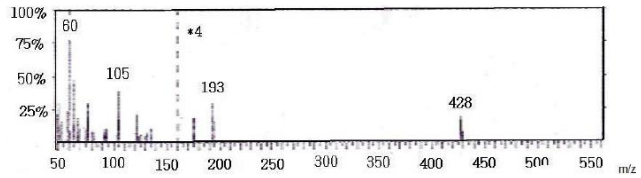

Figure 4 : Mass spectrum of azelastine alkaline degradant

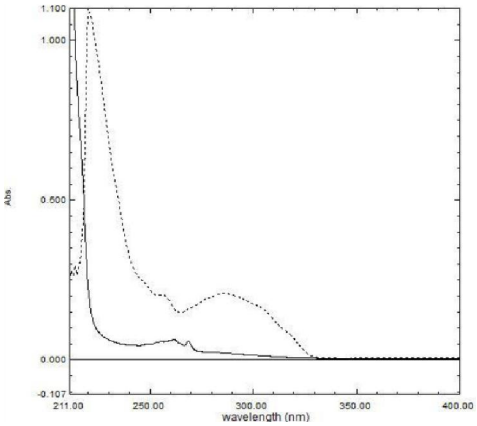

Figure 5 : Zero-order absorption spectra of azelastine hydrochloride, 10 µg ml^{-1} (……) and its alkaline degradant, 50 µg ml^{-1} (——)in methanol.

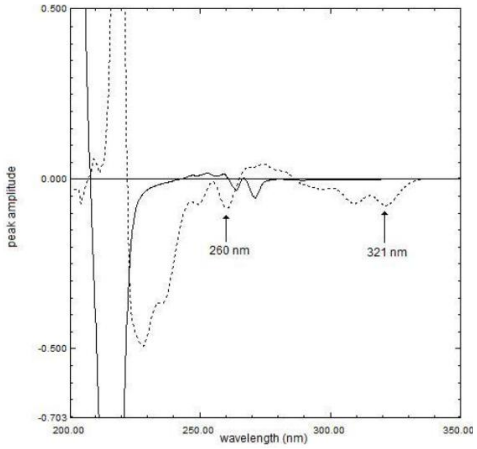

Figure 6 : First-derivative absorption spectra of azelastine hydrochloride, 10 µg ml^{-1} (…..) and its alkaline degradant. , 50 µg ml^{-1} (——) in methanol.

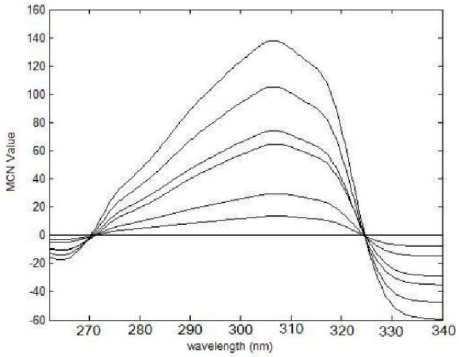

Figure 7 : First-derivative of ratio spectra of 10-90 µg ml^{-1} azelastine hydrochloride using the spectrum of 20 µg ml^{-1} of its alkaline degradant as divisor.

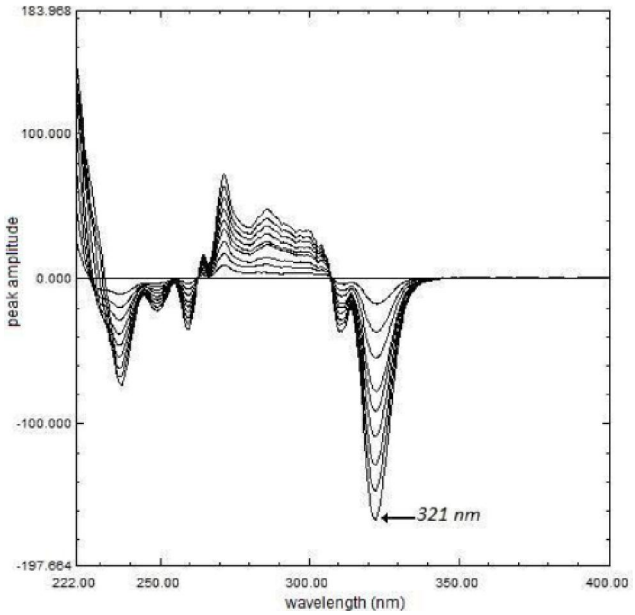

Figure 8 : Mean centered ratio spectra of AZT (10-90 µg mL^{-1}) using the spectrum of 20 µg mL^{-1} of its alkaline degradant as a divisor and methanol as blank.

TABLE 1 : Determination of azelastine hydrochloride in laboratory– prepared mixtures by the proposed spectrophotometric methods.

			D_1 Method		DD_1 Method	MCN
	Conc. Taken (µg ml^{-1}) AZT	Conc. Taken (µg ml^{-1}) Degradant	260 nm	321 nm	321 nm	306.4 nm
			Recovery %	Recovery %	Recovery %	Recovery %
1	10.00	90.00	98.00	96.70	100.10	102.00
2	20.00	80.00	100.50	100.55	100.25	99.15
3	30.00	70.00	98.37	98.57	102.50	100.13
4	40.00	60.00	101.98	100.55	101.28	99.73
5	50.00	50.00	99.62	101.34	99.76	100.10
6	60.00	40.00	99.33	99.07	99.58	100.37
7	70.00	30.00	98.73	98.73	100.01	99.73
8	80.00	20.00	99.39	99.73	100.31	100.33
9	90.00	10.00	99.46	99.51	100.44	100.77
Mean ± SD%			99.49 ±1.190	99.42 ± 1.372	100.47±0.901	100.26±0.802

*** Average of 4 determinations**

TABLE 2 : System suitability parameters for the determination of azelastine HCl in presence of its alkaline degradant by HPLC method.

Parameters	Azelastine hydrochloride	Reference value
Resolution (R)	6.58	R> 0.8
Separation factor (α)	1.96	>1
Capacity factor (K)	8.9	1-10 acceptable
Number of theoretical plates (N)	2173.86	increases with efficiency of the separation
Tailing factor (T)	1.25	T= 1 for a typical symmetric peak
Height equivalent to one theoretical plate (H)	0.04	The smaller the value, the higher the column efficiency

TABLE 3 : Application of standard addition technique for the analysis of azelastine hydrochloride in azelast eye drops* by the proposed spectrophotometric and chromatographic methods.

Method	Found**%	Pure added***	Pure found**	Recovery of added%
D_1 At 260 nm	99.18 ± 0.920	20	19.56	97.80
		30	29.57	98.57
		40	40.00	100.00
Mean ± S.D. %				98.79± 1.117
D_1 At 321nm	100.01 ± 1.420	20	20.36	101.80
		30	29.54	98.47
		40	39.66	99.15
Mean ± S.D. %				99.81 ± 1.761
DD_1 At 321nm	99.43 ± 1.086	20	19.96	99.80
		30	30.00	100.00
		40	40.08	100.20
Mean ± S.D. %				100.00 ± 0.200
MCN At 306.4 nm	99.23 ± 0.403	20	20.20	101.00
		30	30.56	101.87
		40	39.79	99.48

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Method	Found**%	Pure added***	Pure found**	Recovery of added%
Mean ± S.D. %				100.78 ± 1.211
		5	5.02	100.40
Densitometry	101.58± 0.697	10	9.98	99.80
		15	14.76	98.40
Mean ±S.D%				99.53±1.026
		15	14.92	99.47
HPLC	100.23±1.237	20	20.27	101.35
		25	24.96	99.84
Mean ±S.D%				100.22±0.997

*** B.No 16209; ** Average of 4 determinations; *** µg spot^{-1} for densitomertry and µg ml^{-1} for D_1, DD_1, MCN and HPLC methods**

TABLE 4 : Statistical comparison of the results obtained by the proposed methods and the reported method for determination of pure azelastine hydrochloride.

Item	D_1		DD_1	MCN	TLC	HPC	Manufacturer's Method**
	260 nm	321 nm	321 nm	306.4 nm			
Mean	99.99	100.19	100.04	100.13	100.01	100.24	99.26
SD	1.409	1.192	0.672	0.662	1.146	1.418	1.11
RSD%	1.409	1.190	0.672	0.661	1.146	1.415	1.12
N	6	6	6	6	5	6	5
Variance	1.985	1.421	0.452	0.438	1.313	2.011	1.232
Student's t-test	0.938 (2.262)*	1.328 (2.262)*	1.441 (2.262)*	1.616 (2.262)*	1.051 (2.306)*	1.254 (2.262)*	----------
F-value	1.611 (4.95)*	1.153 (4.95)*	2.726 (4.39)*	2.813 (4.39)*	1.066 (5.05)*	1.632 (4.95)*	----------

*** The values in parentheses correspond to the theoretical values of t and F at P = 0.05; ** Manufacturer's UV measurement at λ_{max} 285 nm in 0.1 M HCl.**

TABLE 5 : Assay validation sheet for the proposed methods for the azelastine hydrochloride in presence of its alkaline degradant.

Parameters	Proposed method					
	D_1		DD_1	MCN	HPLC	TLC
linearity	260 nm	321 nm	321 nm	306.4 nm		
Slope	-0.0104	-0.0091	-1.9827	1.7301	1.9978 x10^4	0.7251 x10^3
Intercept	0.0078	0.0056	1.568	0.2347	-1.1371 x10^4	9.5253 x10^3
Correlation Coefficient (r)	0.9998	0.9998	0.9999	0.9999	0.9997	0.9997
Range	10-90 µg mL^{-1}	10-90 µg mL^{-1}	10-90 µg mL^{-1}	10-90 µg mL^{-1}	5-30 µg mL^{-1}	10-30 µg spot^{-1}
Accuracy (Mean ± S.D)	99.99±1.409	100.19±1.192	100.04±0.672	100.13± 0.662	100.24±1.418	100.01± 1.146
Precision (RSD%)						
Repeatability [a]	1.0658	0.8944	0.9299	0.8183	1.2607	0.2844
Intermediate precision [b]	1.2680	0.8414	1.0583	0.9919	1.0028	1.2383
Specificity and Selectivity	99.49 ±1.190	99.42 ± 1.372	100.47±0.901	100.26±0.802	————	————

[a,b] Intraday and interday, relative standard deviations for determination of samples of (20 and 30 µg ml^{-1}) and (15 and 20 µg ml^{-1}) of azelastine HCl for spectrophotometric and chromatographic methods respectively.

Spectrophotometric techniques were investigated for this purpose to solve the problem of overlapping absorption spectra of AZT and its alkaline degradant Figure (5). Figures (6-8) show that D_1, DD_1 and MCN methods can be used. In D_1 method, AZT can be determined at 260 and 321 nm where its degradant has zero contribution, in the DD_1 method, AZT can be determined by measuring the peak amplitude of the first derivative of ratio spectra at 321 nm, while in MCN method, the peak amplitude for mean centered spectra can be determined at 306.4 nm.

All methods were successfully applied for the determination of AZT in lab mixture containing different proportion from its alkaline degradant, indicating the specificity of the methods, TABLE (1).

Chromatographic methods were also investigated and it was found that AZT can be separated from its alkaline degradant using silica gel plates and acetonitrile: water: acetic acid (8:2:1, by volume) as a developing solvent. The R_f values were 0.81 and 0.25 for AZT and its alkaline degradant respectively. The spots corresponding to AZT can thus be scanned at 254 nm.

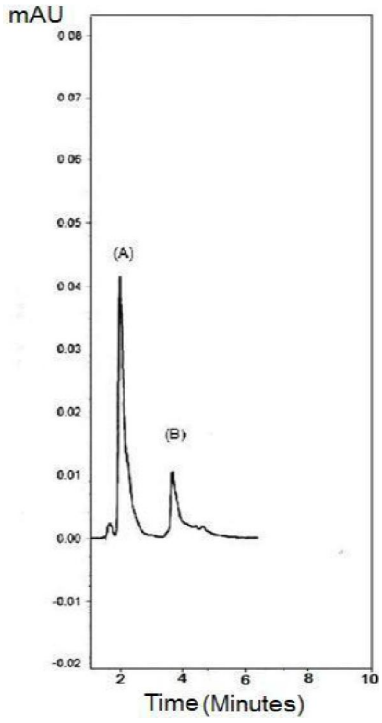

Figure 9 : HPLC chromatogram for (A) Azelastine hydrochloride (30 µg ml^{-1}), (B) Its alkaline degradant (70 µg ml^{-1}) using C18 column, 5 mM ammonium acetate (pH=3.2): acetonitrile (40: 60, v/v) as the mobile phase and UV detection at 253 nm.

AZT could also be separated from its alkaline degradant by HPLC using C_{18} column and 5mM ammonium acetate (pH 3.2): acetonitrile (40:60, v/v) as the mobile phase with UV detection at 253 nm. The average retention times were 1.98 and 3.69 minute for AZT and its alkaline degradant, Figure (8).

The system suitability parameters were calculated. The result shows in TABLE (2) show that the value obtained are conforming to reference values indicating good resolution of AZT and its alkaline degradant.

All the proposed methods were successfully applied for the determination of AZT in pharmaceutical form. The validity of the methods was further assessed by application of the standard addition technique, TABLE (3).

A statistical comparison of the results obtained by the proposed methods and the manufacturer procedure that depends on UV spectrophotometric determination of azelastine hydrochloride [16] for pure drugs is shown in TABLE (4). Calculated t- and F-values are less than the tabulated values, which reveals that there is no significant difference with respect to accuracy and precision between the proposed methods and the manufacturer's procedure.

The results of assay validation of the proposed methods show that they are accurate, precise, specific, and rugged according to the RSD values of intraday and interday determinations TABLE (5).

CONCLUSION

The aim of this work is to develop simple methods for the determinations of azelastine hydrochloride in presence of its alkaline degradant. The derivative, derivative ratio, mean centering TLC and HPLC methods could be applied to the determination of azelastine hydrochloride in presence of its alkaline degradant either in their pure powder form or in their pharmaceutical formulation. The results demonstrate the usefulness of the methods, which are simple, sensitive, precise, accurate, inexpensive and non-polluting. So, the proposed methods could be used in routine and quality control analysis of azelastine hydrochloride.

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