Original article

Development and validation of HPTLC densitometric method for simultaneous determination of antazoline hydrochloride and tetryzoline hydrochloride in eye drop and its application as stability indicator

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Abstract:

A sensitive, selective, precise, accurate and stability indicating high-performance thin layer chromatographic (HPTLC)-densitometric method for analysis of antazoline hydrochloride and tetryzoline hydrochloride was developed. The method employed HPTLC aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of ethyl acetate:methanol:ammonia (10:10:1, v/v/v). Densitometric analysis of drugs was carried out in the absorbance mode at 216 nm. This system gave compact spots for both antazoline hydrochloride (R_f 0.60 ± 0.03) and tetryzoline hydrochloride (R_f 0.31 ± 0.03). Both drugs were subjected to stress test conditions like acid/alkali hydrolysis, oxidation by hydrogen peroxide, dry heat treatment and photo degradation. The spots for products of degradation were well resolved from the spots of respective intact drugs. The linear regression data for the calibration plots showed good linear relationship with r^2 of 0.9979 and 0.9990 in the concentration range of 200-1800 ng/band for antazoline hydrochloride and 160-1440 ng/band for tetryzoline hydrochloride, respectively. The results indicated that the drugs are susceptible to degradation, to different extent under the different conditions.

Keywords: Antazoline HCl; Tetryzoline HCl; HPTLC-densitometry; Stability indicating assay

Introduction

Allergic conjunctivitis is the most common clinical form of ocular allergy, and the underlying immune reaction tends to be mediated by IgE. The usual treatment of allergic conjunctivitis comprises non-specific measures such as the application of cold dressings, artificial tears and the avoidance of allergens. However, these measures are typically ineffective or not very practical, and pharmacological treatment normally proves necessary. Topical and systemic antihistamines, topical vasoconstrictors, topical non-steroidal anti-inflammatory drugs (NSAIDs) and topical corticoids can be used. Antazoline is a short-acting moderately-sedative antihistamine (with low anticholinergic effect) given by mouth/topically to relieve the symptoms of allergic reaction. It is less irritating than the other antihistamines, but it produces their characteristic side effects, such as drowsiness, dizziness, and incoordination. Tetryzoline (tetrahydrozoline) is a sympathomimetic drug (alpha agonist) that constricts blood vessels and is used as nasal and conjunctival decongestant [1, 2].

Antazoline HCl is a 4,5-dihydro-N-phenyl-N-(phenylmethyl)-1H-imidazole-2-methanamine hydrochloride. It is a white or almost white, crystalline powder, sparingly soluble in water (1 in 40), soluble in alcohol (1 in 25), slightly soluble in methylene chloride and practically insoluble in benzene, chloroform and ether. It melts at about 240°C (237-241°C) with decomposition. Its molecular formula is $C_{17}H_{19}N_3$, HCl and molecular weight is 301.8. Tetryzoline HCl is a 4,5-dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl)-1H-imidazole hydrochloride. It is a white or almost white, crystalline powder and melts between (256-257°C), with decomposition. It is freely soluble in water (1 in 3.5), in anhydrous ethanol (1 in 7.5) and in ethanol (96 per cent), and practically insoluble in acetone. It has a molecular formula of $C_{13}H_{16}N_2$, HCl and molecular weight of 236.7 [3, 4].

A GC-MS was developed for analysis of tetryzoline in body fluids [5]. A HPLC method was described for the determination of benzalkonium chloride homologues and naphazoline nitrate or tetryzoline hydrochloride in ophthalmic and nasal solutions [6]. Reversed-phase (RP) ion pair HPLC method was described for the determination of antazoline phosphate and tetryzoline hydrochloride in ophthalmic solution [7]. A HPLC method has been developed for the simultaneous determination of ofloxacin, tetryzoline hydrochloride, and prednisolone acetate in ophthalmic suspension using propylparaben as the internal standard [8]. Second derivative UV spectrophotometry and RP HPLC methods were described for the quantitative determination of tetryzoline hydrochloride and fluorometholone in eye drops (efemoline) [9]. A zero-crossing first-derivative spectrophotometric method was applied for the simultaneous determination of naphazoline hydrochloride and antazoline phosphate in eye drops [10]. A spectrophotometric determination of antazoline salts was developed by improving the ceric sulphate procedure. Replacement of water with acetic acid for the preparation of all assay solutions permitted reproducible measurements of the chromogen that absorbed at 505 nm [11]. The dc-polarographic investigation of antazoline hydrochloride in aqueous acidic media was described [12]. According to British Pharmacopeia 2009, antazoline HCl was assayed by dissolving in alcohol and titrating with alcoholic potassium hydroxide in the presence of phenolphthalein. Related substance was examined by thin-layer chromatography using silica gel GF254 as the coating substance and diethyl amine-methanol-ethyl acetate as a mobile phase. Tetryzoline HCl was assayed by dissolving in mixture of anhydrous acetic acid and acetic anhydride and titrating it with perchloric acid where the end point was detected potentiometrically [3]. In US Pharmacopeia/National Formulary 2007 [13], antazoline phosphate was assayed after dissolving in glacial acetic acid and then titrating potentiometrically with perchloric acid. Identification test was done by thin layer chromatography using ethyl acetate: methanol: diethyl amine as a mobile phase. Assay of tetryzoline HCl was done by dissolving in glacial acetic acid-acetic anhydride mixture and then titrating with perchloric acid. The assay of tetryzoline HCl nasal solution utilized spectrophotometric method (absorbance measured at 570 nm) after treating with oxidized nitroprusside reagent. Ophthalmic solution of tetryzoline

HCl was determined spectrophotometrically at 415 nm after treating with bromophenol blue sodium salt and extracting with chloroform [13].

High performance thin-layer chromatography (HPTLC) method is a powerful analytical technique due to its merits of reliability, simplicity, reproducibility and speed. HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents and biomacromolecules. Solvent consumption per sample basis with HPTLC is only 5% of that with LC and it requires minimum sample clean up i.e., direct application of suspensions, dirty or turbid samples. Thus this method becomes more economical and less hazardous. A number of samples can be analysed in a single run, reducing the analysis time; it has high sample throughput. HPTLC allows visualisation of samples at every stage of analysis; moreover post chromatographic derivatization is easy. HPTLC has no limitation on the choice of the mobile phase and unlike HPLC, mobile phases having pH 8 and above can be employed. Therefore, by comparison with HPLC, HPTLC still has the advantage of being a rapid, reliable and economical analysis method. In recent years, HPTLC has been improved to incorporate the following features: HPTLC grade stationary phase, automated sample application devices, controlled development environment, automated developing chamber, computer-controlled densitometry and quantitation, and fully validated procedures. These features make the method not only convenient, fast, robust and cost-efficient, but also reproducible, accurate and reliable [14].

The objective of this study was to develop and validate HPTLC-densitometric method for simultaneous determination of antazoline HCl and tetryzoline HCl in eye drops and to test the method as stability indicator.

Experimental

Equipments and instruments

Computerized Camag HPTLC system consisting of a Camag Linomat V semi-automatic spotting device connected to a nitrogen tank and WinCATS 4 software version 4.05, a Camag TLC scanner III densitometer equipped with mercury, tungsten and deuterium lamp driven by the same WinCATS 4 software, a Camag glass twin-trough (20 cm × 10 cm) development chamber (Camag, Muttenz, Switzerland), and a Camag 100 µl HPTLC sample syringe (Camag, Hamilton, Switzerland), different size pipettes, volumetric flasks, digital analytical balance and ruler.

Chemicals and solvents

Antazoline hydrochloride (purity, 99.9%) and tetryzoline hydrochloride (purity, 99.7%) reference standards (Sigma-Aldrich, Germany), HPTLC plates (silica gel 60 F_{254}, aluminium backing) (Camag, Switzerland) were used. Analytical grades of ethyl acetate, methanol and ammonia (35%) (BDH, England) were used.

Preparation of standard solutions

Stock solutions of the reference standards containing 1 mg/ml of antazoline hydrochloride and 0.8 mg/ml of tetryzoline hydrochloride were prepared. Working standard solutions were prepared by dilution of 10 ml of the stock solutions ten times with methanol to give a concentration of 100 µg/ml for antazoline hydrochloride and 80 µg/ml for tetryzoline hydrochloride.

Preparation of sample solutions

The contents of five bottles were mixed and 10 ml of the eye drop, equivalent to 5 mg of antazoline hydrochloride and 4 mg of tetryzoline hydrochloride, was diluted to 50 ml with methanol in volumetric flask to get a concentration of 100 µg/ml for antazoline hydrochloride and 80 µg/ml for tetryzoline hydrochloride [15].

Chromatographic conditions

The mobile phase (21 ml per run) consisting of ethylacetate:methanol:ammonia (10:10:1 v/v/v) was used for method development. Samples were spotted in the form of bands of width 6 mm (2, 4, 6, 8, 10, 12, 14, 16 and 18 µl were applied for linearity study). A constant application rate of 0.1 µl/s was employed. Linear ascending development was carried out in a twin trough glass chamber (20 cm × 10 cm) saturated with the mobile phase. The optimized chamber saturation time was 30 min at room temperature of 22 ± 2°C and relative

humidity of 60 ± 5%. The mobile phase was allowed to cover a distance of 8 cm and that took approximately 35 min. Subsequent to development, the plates were dried with a help of hair dryer. Densitometric scanning was performed in the absorbance mode at 216 nm for both antazoline hydrochloride and tetryzoline hydrochloride. The slit dimension of the scanner was kept at 6 mm × 0.30 mm and 20 mm/s scanning speed was employed. Concentrations of the compounds chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas versus concentration with linear regression using Microsoft excel 2003.

Method validation

The analytical method was validated in compliance with ICH guidelines [16, 17]. The following parameters were performed for validation:

Linearity

Linearity of the peak area response was determined by making six measurements in the range of 200-1800 ng/band for antazoline hydrochloride and 160-1440 ng/band for tetryzoline hydrochloride [18].

Accuracy

The recovery studies were carried out by applying the proposed method on the drug sample to which known amount of antazoline hydrochloride and tetryzoline hydrochloride standard corresponding to 80, 100 and 120% of label claim had been added (standard addition method). At each level of the amount added three determinations were performed and the results obtained were compared with the theoretical amounts [19].

Precision

The repeatability of sample application and the repeatability of measurement of peak area were performed by the proposed method for system precision studies to determine variations due to the instrument. The repeatability of sample application was carried out by making six measurements on a single stock solution (for antazoline = 1000 ng/band; for tetryzoline HCl = 800 ng/band). Repeatability of measurement of peak area was determined by scanning of the developed spot (for antazoline = 1000 ng/band; for tetryzoline HCl = 800 ng/band) six times without changing the plate position. In both cases, i.e. the repeatability of sample application and the repeatability of measurement of the peak area, the coefficient of variation of measurement of the peak area were taken to evaluate the system precision.

Method repeatability was carried out by repeating the assay six times in the same day for intra-day precision and by repeating the assay on three different days, three times on each day, for intermediate precision (inter-day precision) to determine variations arising due to the method itself. The intra-day and inter-day variation for determination of the two drugs was carried out at three different concentration levels 600, 800, 1000 ng/band for antazoline HCl and 480, 640, 800 ng/band for tetryzoline HCl. In both cases the coefficient of variation of the peak area of the spots was used to evaluate method precision. Inter-analyst variation was performed by three different analysts in triplicate at three concentration levels (600, 800, 1000 ng/band for antazoline HCl, and 480, 640, 800 ng/band for tetryzoline HCl). The coefficient of variation (CV) of peak area was calculated for results of each analyst.

Limit of detection and limit of quantification

Determination of the detection and quantitation limits was performed based on the standard deviation of the response and the slope. The slope was estimated from the calibration curve of the analyte and the estimate of the standard deviation was carried out from the standard deviation of *y*-intercepts of regression lines. Signal/noise ratios of 3.3:1 and 10:1 were considered as limit of detection and limit of quantification, respectively.

Specificity

Peak purity of both antazoline HCl and tetryzoline HCl was assessed to evaluate the specificity of the method. The spots of dosage forms were scanned at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions before method validation. Correlation coefficients of these spectra were calculated. The spectra of dosage form and reference standards were also compared for both studied drugs.

Robustness

Effects of small but deliberate changes in the

mobile phase composition, total volume of mobile phase, time between application of the sample and insertion into the development chamber and time between chromatograms developing and scanning were examined. Mobile phases having different compositions of ethylacetate:methanol:ammonia *viz* (9:10:1); (11:10:1); (10:9:1); (10:11:1); (10:10:0.9) and (10:10:1.1) were used to develop chromatograms.

The amount of mobile phase was varied in the range of ± 10% and both time variations: time from spotting to developing and from developing to scanning was studied at zero, 20 and 40 min. For each of the effects concentration levels of 600, 800 and 1000 ng/band for antazoline HCl, and 480, 640 and 800 ng/band for tetryzoline HCl, and three determinations were performed to study the robustness of the method. The relative standard deviations of the peak area of the spots were taken to document the robustness of the method.

Forced degradation study

Antazoline HCl (25 mg) and tetryzoline HCl (20 mg) were dissolved in 25 ml methanol to give stock mixture solution containing 1 mg/ml for antazoline HCl and 0.8 mg/ml for tetryzoline HCl. For acid and base-induced degradation study, 5 ml each of the stock mixture solution was mixed separately with 5 ml of methanolic solution of 0.1 M HCl and 0.1 M NaOH. The resulting solutions were kept at room temperature for 8 h or refluxed at 75°C for 8 h.

For hydrogen peroxide (H_2O_2)-induced degradation study, 5 ml of the stock mixture solution was mixed with 5 ml of methanolic solution of hydrogen peroxide (3.0% v/v). The resulting solution was kept for 8 h at room temperature in dark to exclude the possible degradative effect of light. The dry heat-induced degradation study was performed by keeping drug samples in solid state in oven at 80°C for 8 h. The 25 mg of heat-treated antazoline HCL and 20 mg of heat-treated tetryzoline HCl were dissolved in 25 ml of methanol prior to analysis. For the light-induced degradation study the experiment was performed similar to the dry heat induced degradation study except that the drug powders were exposed to direct sunlight for 72 h.

Results and Discussion

Method optimization

After evaluation of various solvent systems to arrive at an optimum resolution of antazoline HCl and tetryzoline HCl, the solvent system consisting of ethylacetate: methanol:ammonia (10:10:1, v/v/v) gave dense, compact and well separated spots with R_f value of 0.31 ± 0.03 and 0.60 ± 0.03 for tetryzoline HCl and antazoline HCl, respectively.

Since both drugs exist in hydrochloride salt form, ammonia was needed to release the free bases. Polar solvent like methanol was required to overcome the strong interaction between the two drugs (basic) and the highly polar silica gel (acidic). Chamber saturation was studied for 10, 15, 20, 25 and 30 min. It was observed that pre-saturation of the chamber with the mobile phase for 30 min ensured good reproducibility and symmetrical peak shape. Spectra overlay of the two drugs showed that they crossed each other at 216 nm and 224 nm. With the objective of enhancing sensitivity, densitometric evaluation was performed at 216 nm for both drugs.

Method validation

Linearity

The relationship between the concentration of each analyte and peak area of the spots was investigated. The characteristic parameters of the linear regression equation of the two drugs are shown in Table 1. The correlation coefficients (r) were found to be 0.9989 and 0.9995 for antazoline HCl and tetryzoline HCl, respectively, indicating high correlation.

Accuracy

The recoveries ranged from 98.96% to 101.11% (99.78 ± 1.01%) for antazoline HCl and from 98.24% to 101.69% (99.79 ± 1.49%) for tetryzoline HCl. These satisfactory recoveries with small coefficient of variation (< 2%, for both drugs) indicate that the method provides sufficient accuracy [20].

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of coefficient of variation and were found to be 0.12 and 0.73 for antazoline HCl and 0.37 and 0.63 tetryzoline

HCl, respectively. The results insured reproducible performance of the instrument. The coefficient of variation values showed that the proposed method provided acceptable intra-day and inter-day variation of antazoline HCl and tetryzoline HCl, indicating the reproducible performance of the method. The variation results between three analysts were presented in Table 2. The low values of CV indicated a good agreement among the results of the analysts. As depicted in Tables 2 and 3, the coefficient of variation less than 2% suggests system suitability and precision of the developed method [20].

Limit of detection and limit of quantification

The limit of detection and limit of quantification of the proposed method were calculated according to 3.3σ/S and 10σ/S criteria, respectively, where σ is the standard deviation of y-intercepts of regression lines (n=6) of the sample and S is the slope of the corresponding calibration curve. The limits of detection of antazoline HCl and tetryzoline HCl were found to be 63.95 and 36.01 ng/band, respectively. The limit of quantification of antazoline HCl and tetryzoline HCl were 193.79 and 109.11 ng/band, respectively. Summary of method validation parameters is presented in Table 4.

Specificity

The peak purity test of antazoline HCl and tetryzoline HCl spots were assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot. The results of spectral comparison for antazoline HCl were found to be 0.9995 and 0.9996 at peak start-peak apex and at peak apex-peak end, respectively. Similarly, for tetryzoline HCl the results were 0.9997 and 0.9998. The closeness of peak purity values to 1 indicates that the spots were only attributed to a single compound. The UV spectra comparison of the spots of the standards and dosage forms for the study substances are presented in Figure 1.

Analysis of the marketed formulation

The proposed method was applied to the determination of antazoline HCl and tetryzoline HCl in eye drops of Spersallerg®. Six replicate determinations were made. Acceptable results were obtained for both drugs and were in a good agreement with the label claims (95 to 105% for both drugs). The drug content was found to be 99.58% ± 1.03 (coefficient of variation = 1.04) and 103.78% ± 0.99 (coefficient of variation = 0.95) for antazoline HCl and tetryzoline HCl, respectively. The low values of coefficient of variation indicated the suitability of this method for routine analysis of the two drugs in pharmaceutical dosage forms.

Stability of sample solution

The time the sample is left in solution prior to chromatographic development can influence the stability of drugs and are required to be investigated. Solutions of different concentrations 100 ng/µl for antazoline HCl and 80 ng/µl for tetryzoline HCl were prepared and stored at room temperature for 1.0, 4.0, 8.0 and 24 h respectively.

They were then applied on the HPTLC plate, after development the densitograms were evaluated to check out the presence of additional spots. So there was no indication of compound instability during the analysis. Also Table 5 shows a coefficient of variation (CV) value of less than 2% which indicates that the solution is stable for 24 h.

Table 1 Characteristic parameters for the linear regression equations obtained from the HPTLC- densitometric method for the studied drugs (n=6)

Parameters	Antazoline HCl	Tetryzoline HCl
Linearity rage (ng/band)	200-1800	160-1440
Slope ± S.D.	3.1869 ± 0.0549	2.6199 ± 0.0317
Intercept ± S.D.	886.8447 ± 61.7604	217.0881 ± 28.5852
Determination coefficient (r^2)	0.9979	0.9990
Correlation coefficient (r)	0.9989	0.9995

All calculations were done under 95% confidence limit.

Table 2 Result of variation between analysts (n = 3)

Analyst	Concentration level of the two drugs																	
	Antazoline hydrochloride (ng/band)									Tetryzoline hydrochloride (ng/band)								
	600			800			1000			480			640			800		
	MPA	SD	CV	MPA	SD	CV	MPA	SD	CV	MPA	SD	CV	MPA	SD	CV	MPA	SD	CV
1	2724.33	15.24	0.57	3456.63	29.69	0.86	4167.59	24.17	0.58	1420.87	4.14	0.29	1936.18	3.95	0.20	2366.43	14.48	0.61
2	2734.67	14.23	0.52	3487.21	4.80	0.14	4204.11	5.19	0.12	1439.42	10.71	0.74	1932.33	13.61	0.70	2371.54	2.54	0.11
3	2721.60	7.07	0.26	3469.99	9.89	0.29	4186.32	6.06	0.14	1425.11	1.93	0.14	1929.96	7.90	0.41	2363.64	10.14	0.43

MPA = mean peak area; SD = standard deviation; CV = coefficient of variation.

Table 3 Robustness testing of the developed method (n = 3)

Parameter	Concentration of AHC (ng/band)									Concentration of THC (ng/band)								
	600			800			1000			480			640			800		
	Mean area	SD	CV	Mean area	SD	CV	Mean area	SD	CV	Mean area	SD	CV	Mean area	SD	CV	Mean area	SD	CV
MPC	2840.72	10.56	0.37	3441.36	16.80	0.49	4147.23	6.11	0.15	1443.90	12.70	0.88	1934.56	9.23	0.48	2344.39	5.50	0.23
AOMP	2850.47	24.67	0.87	3444.10	11.30	0.33	4147.43	32.07	0.77	1438.70	1.15	0.08	1939.02	13.10	0.68	2335.81	13.98	0.60
TFSTD	2875.59	12.45	0.43	3441.82	13.77	0.40	4151.67	13.92	0.34	1454.36	13.77	0.95	1960.50	7.80	0.40	2322.03	7.83	0.34
TFDTS	2850.35	13.03	0.46	3435.65	10.15	0.30	4151.81	19.83	0.48	1431.08	3.63	0.25	1930.25	11.74	0.60	2362.80	4.59	0.19

AHC = antazoline hydrochloride; THC = tetryzoline hydrochloride; SD = standard deviation; CV = coefficient of variation; MPC = mobile phase composition;
AOMP = amount of mobile phase; TFSTD = time from spotting to development; TFDTS = time from development to scanning.

Table 4 Summary of method validation parameters

Parameters	Antazoline HCl	Tetryzoline HCl
Linearity range (ng/band)	200-1800	160-1440
Correlation coefficient	0.9989	0.9995
Limit of detection (ng/band)	63.95	36.01
Limit of quantification (ng/band)	193.79	109.11
Recovery (mean ± S.D.)	99.78 ± 1.01	99.78 ± 1.49
Precision (C.V.)		
Repeatability of application (n=6)	0.12	0.01
Repeatability of measurement (n=6)	0.37	0.63
Intra-day (n=6)	0.60	0.71
Inter-day (n=3)	0.12	0.34
Robustness	Robust	Robust

S.D* = standard deviation; C.V** = coefficient of variation.

Table 5 Stability of antazoline HCl and tetryzoline HCl in solution (n = 6)

Drug	Time (h)	Amount (ng)	Mean area (Au)	S.D.	C.V.
Antazoline HCl	0	800	3425.64	26.25	0.77
	1	800	3417.87	27.94	0.82
	4	800	3412.31	23.79	0.70
	8	800	3403.69	30.20	0.89
	24	800	3401.27	27.94	0.82
Tetryzoline HCl	0	640	1968.29	16.59	0.84
	1	640	1961.26	13.63	0.69
	4	640	1950.93	17.65	0.90
	8	640	1947.07	18.45	0.95
	24	640	1940.84	17.74	0.91

SD = standard deviation; CV = coefficient of variation.

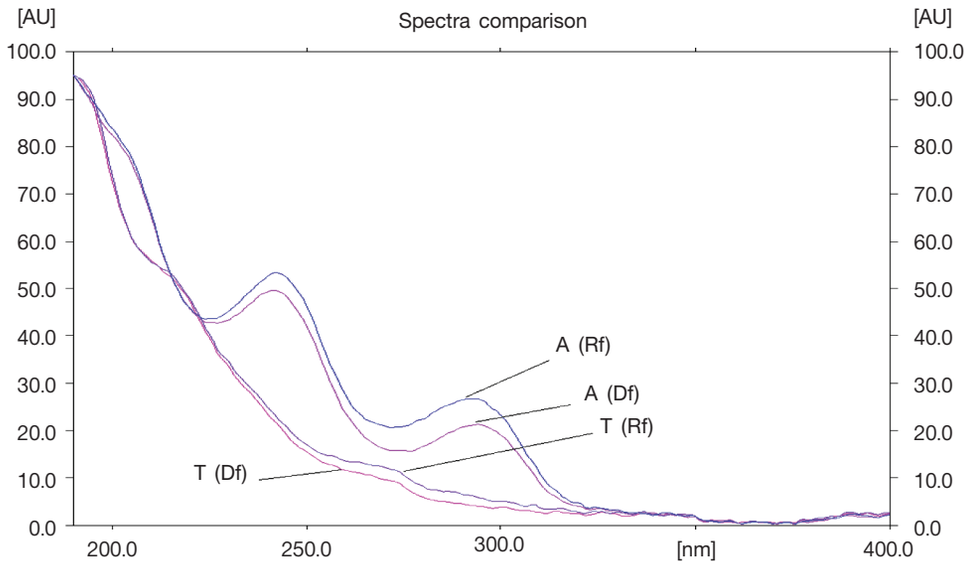

Figure 1 UV spectra comparison of the spots of the standards (R_f) and dosage forms (D_f) for antazoline HCl (A) and tetryzoline HCl (T)

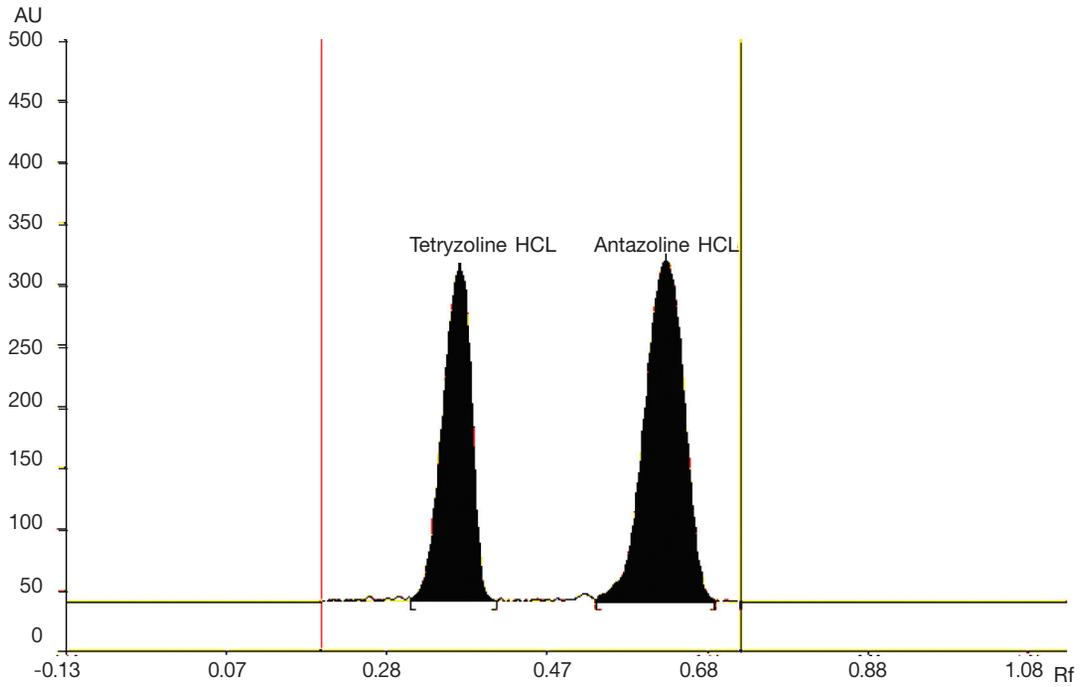

Figure 2 Densitogram of untreated synthetic mixture sample

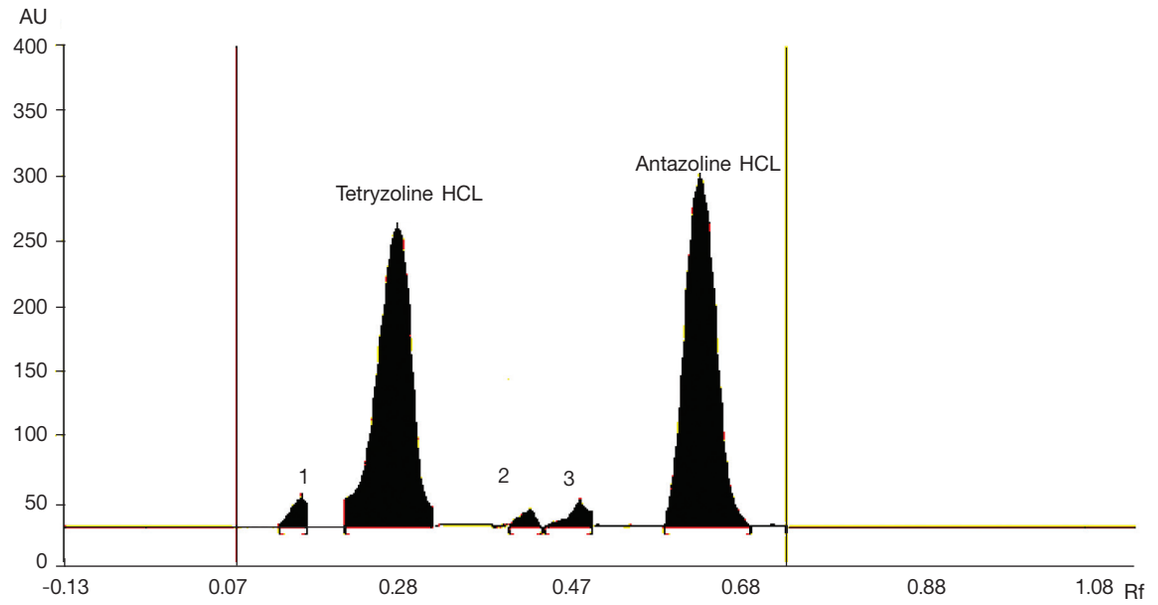

Figure 3 Densitogram of acid-treated synthetic mixture sample at 75°C

Forced degradation study for antazoline HCl and tetryzoline HCl

Acid-treated tetryzoline HCl showed additional peaks at R_f values of 0.13 and 0.60 whereas acid-treated antazoline HCl gave extra peaks at R_f values of 0.42 and 0.48. The densitograms of acid-treated sample (synthetic mixture) at room temperature did not show extra peaks. But at 75°C, it showed additional peaks at R_f values of 0.13, 0.42 and 0.48 (Figure 3). The degradation product that appeared in acid-treated tetryzoline HCl (R_f = 0.60) had a similar R_f as that of antazoline HCl. This would give a single peak for degradation product of tetryzoline HCl and authentic antazoline HCl.

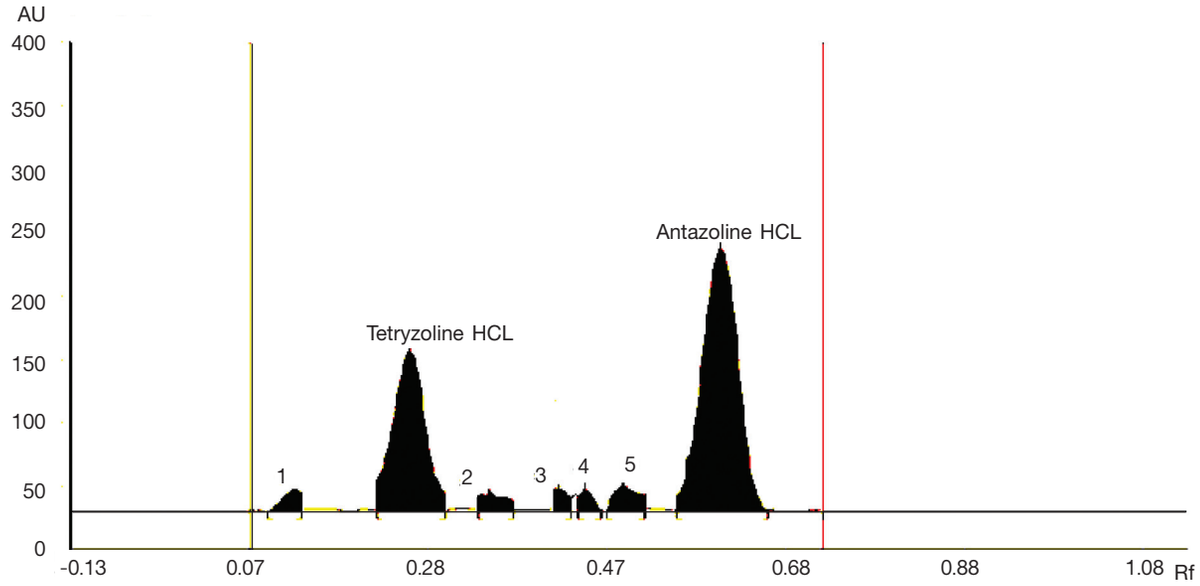

Figure 4 Densitogram of base-treated synthetic mixture sample at 75°C

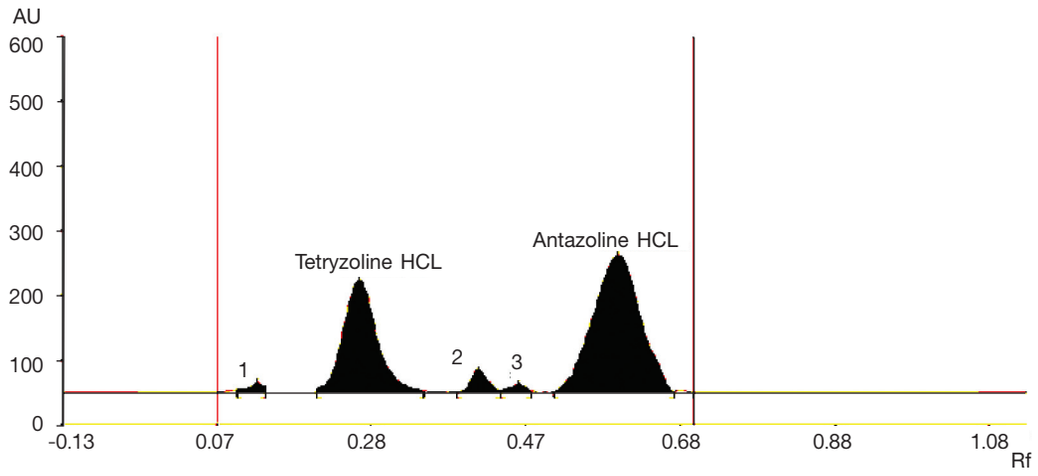

Figure 5 Densitogram of H_2O_2-treated synthetic mixture sample

The spectral pattern of individual drugs and that of synthetic mixture is not exactly similar for the synthetic mixture (Figure 2). The probable reason may be due to overlap of peaks with very close R_f values of components from the different drugs Viz: R_f 0.32 with R_f 0.34; R_f 0.49 with R_f 0.50 and R_f 0.58 and 0.61 with R_f 0.60. Base-treated tetryzoline HCl resulted in new peaks at R_f values of 0.12, 0.34, 0.50, 0.58 and 0.61 while base-degraded antazoline HCl gave additional peaks at R_f values of 0.23, 0.34, 0.38, 0.41, 0.45 and 0.49. The chromatograms of base-treated sample (synthetic mixture) did not give additional peaks at room temperature. On heating to 75°C, extra peaks were generated at R_f values of 0.12, 0.34, 0.41, 0.45 and 0.49 (Figure 4).

Hydrogen peroxide (H_2O_2)-treated tetryzoline HCl produced peaks at R_f values of 0.12, 0.53, 0.60 and 0.62 and H_2O_2 treated antazoline HCl revealed the presence of extra peaks at R_f values of 0.50 and 0.67. Examination of densitograms after treating the sample (synthetic mixture) with hydrogen peroxide revealed the presence of extra peaks at R_f values of 0.12, 0.40 and 0.46 (Figure 5). After heat treatment, peaks were obtained at R_f values of 0.13, 0.50 and 0.57 for tetryzoline HCl and at R_f values of 0.17, 0.25 and 0.44 for antazoline HCl. On treatment of the sample (synthetic mixture)

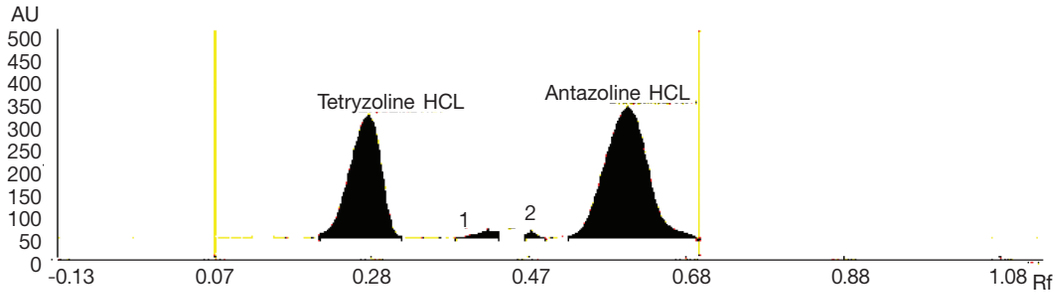

Figure 6 Densitogram of heat-treated synthetic mixture sample

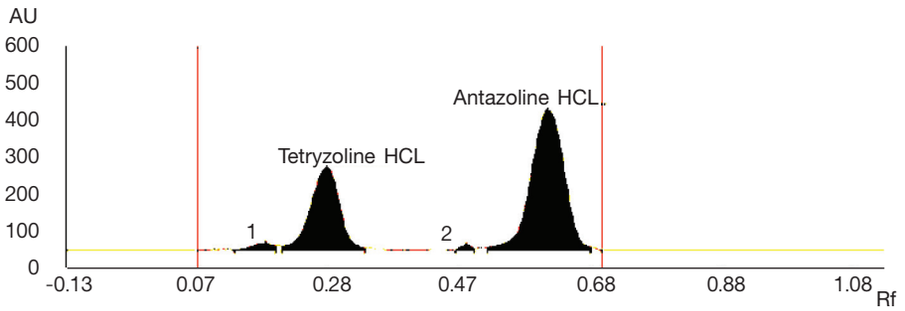

Figure 7 Densitogram of light-treated synthetic mixture sample

with heat, extra peaks were generated at R_f values of 0.40 and 0.49 (Figure 6). Photo-degraded tetryzoline HCl produced peaks at R_f values of 0.52, 0.58 and 0.63 whereas light-treated antazoline HCl gave peaks at R_f values of 0.40, 0.48, 0.64 and 0.69. The chromatograms of light degraded sample (synthetic mixture) produced additional peaks at R_f values of 0.14 and 0.48 (Figure 7).

Conclusion

The developed and validated HPTLC-densitometric method is simple, accurate, precise and specific and thus it could be used for routine analysis of antazoline HCl and tetryzoline HCl in eye drops; the method could also be used as stability-indicating one.

Recommendation

The method should be extended to analysis of antazoline HCl and tetryzoline HCl in different matrices as in the case of biological fluids.

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