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Notes

Stability Study of the Antihistamine Drug Azelastine HCl along with a Kinetic Investigation and the Identification of New Degradation Products

Rania N. El-Shaheny*,† and Koji Yamada****

*Analytical Chemistry Department, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt
**Department of Pharmaceutical Sciences, Graduate School of Biomedical Science, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852–8521, Japan

The first stability-indicating HPLC method was developed and validated for azelastine HCl (AZL). The separation of AZL from its degradation products was achieved on a C_{18} column using acetonitrile-0.04 M phosphate buffer of pH 3.5 (32:68, v/v) as a mobile phase with UV-detection at 210 nm and naftazone as an internal standard. The method was rectilinear over the range of 0.2 – 20.0 μg mL^{-1} with a detection limit of 7.05 ng mL^{-1}. The degradation behavior of AZL was studied under different ICH-recommended stress conditions along with a kinetic investigation; also, degradation products were identified by mass spectrometry. The method was applied for the quality control and stability assessment of AZL in eye drops and nasal spray. The obtained results were favorably compared with those obtained by a comparison method.

Keywords Azelastine HCl, HPLC, stability-indicating, degradation kinetics, mass spectrometry, quality control

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Introduction

Azelastine hydrochloride (AZL), 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl]phthalazin-1(2H)-one hydrochloride,[1] is an antihistamine that blocks histamine H_1-receptor activity, and hinders the release of inflammatory mediators from mast cells. It is used in the symptomatic relief of allergic conditions, including rhinitis and conjunctivitis.[2]

AZL is authorized in the British Pharmacopoeia,[1] which recommends a titrimetric method for its determination in pure form using 0.1 M perchloric acid as a titrant. Scientific literature includes some analytical methods for the determination of AZL in pharmaceutical formulations and/or biological fluids, such as spectrophotometry,[3,4] voltammetry,[5-7] LC/MS-MS,[8-10] HPLC,[11-14] electrokinetic capillary chromatography[10] and TLC.[15] Few reports have mentioned the stability of AZL, including a voltammetric method[7] that determined the drug in presence of its alkaline degradation product using an ion selective electrode and a TLC method[15] for its determination in the presence of acidic and oxidative degradation products. Though, these studies gave helpful information about AZL degradation, it is not sufficient for a complete stability protocol of AZL. So far, the scientific literature lacks any stability-indicating HPLC method for AZL despite its unique advantages and separation power as compared to other analytical techniques. While carrying out a manufacturing process of any formulation it is essential to investigate the presence of degradation products or impurities in the raw materials used for production. These substances may interfere with the solubility of active pharmaceutical ingredients, or affect their safety limits by producing adverse drug reactions or toxicities in the body. These facts initiate the present study to (i) optimize the first stability-indicating HPLC method for AZL, (ii) study its degradation behavior under different ICH-recommended stress conditions,[16,17] and (iii) characterize its degradation products by mass spectrometry.

† To whom correspondence should be addressed.
E-mail: rania_n2010@yahoo.com

Experimental

Instruments

Chromatographic separation was achieved on a Hitachi HPLC system (Tokyo, Japan) equipped with a 655A-11 liquid chromatograph, a high sensitivity series L-4000 H UV-detector, a D-2500 chromato-integrator, a Hitachi LC-organizer and a Rheodyne injector valve with a 20-μL sample loop. An SK Sato pH/mV meter (Sato Keiryoki MFG Co., Ltd., China) was used for pH adjustment. Positive fast atom bombardment mass spectra (FAB^+ MS) were recorded using a JMS DX-303 mass spectrometer (JEOL Ltd., Japan).

Materials

Azelasine HCl (certified purity of 99.85%, Batch #4802304001) was kindly provided by European Egyptian Pharmaceuticals Ind. (Alexandria, Egypt). Naftazone (certified purity of 99.90%, Batch #0301030075), was kindly provided by Alkan Pharma Co. (6th of October City, Egypt). Zalastin® metered dose nasal spray (Batch #3579002), labeled as containing 1 mg mL^{-1} AZL, product of European Egyptian Pharmaceuticals Ind. (Alexandria, Egypt) and Azelast® eye drops (Batch #130157), labeled as containing 0.05 mg mL^{-1} AZL, product of El-Kahira Pharm. & Chem. Ind. Co. (Cairo,

Egypt) were purchased from an Egyptian pharmacy.

Reagents

Purified water was obtained using a Millipore direct-Q 3UV water-purification system (France). All solvents used were of HPLC grade, and chemicals were of extra-pure analytical reagent grade. Acetonitrile was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Orthophosphoric acid (85% w/v) and potassium dihydrogen phosphate were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hydrochloric acid (35 – 37%) and sodium hydroxide were obtained from Chameleon Reagent (Osaka, Japan). Hydrogen peroxide (30% w/v) was obtained from Nacalai Tesque Inc. (Kyoto, Japan).

Standard solutions

A standard solution of AZL containing 1000.0 μg mL^{-1} was prepared in the mobile phase and diluted as appropriate with the same solvent to obtain the working concentration range. A standard solution of naftazone (internal standard, IS) containing 100 μg mL^{-1} was prepared in acetonitrile. Solutions were found to be stable for at least 7 days when kept in a refrigerator at 4°C.

Chromatographic conditions

Separation was performed on a Cosmosil 5C_{18}-MS column (150 mm × 4.6 mm i.d., 5-μm particle size) from Nacalai Tesque, Inc. A mobile phase consisting of acetonitrile-0.04 M phosphate buffer of pH 3.5 (32:68, v/v) was filtered through a 0.45-μm Millipore membrane filter and sonicated for 30 min before use. The flow rate was 1.0 mL min^{-1} and UV-detection was set at 210 nm. Naftazone was used as IS at a final concentration of 10 μg mL^{-1}.

Calculation of kinetic parameters

The first-order degradation rate constants (k) were calculated from the slopes of semi-logarithmic plots of the log $a/(a - x)$ *versus* time, t, in accordance with Eq. (1):[18]

$$kt = 2.303 \log \frac{a}{a-x}. \quad (1)$$

Here, (a) is the initial drug concentration and ($a - x$) the remaining drug concentration.

The half-life time ($t_{1/2}$) for the first-order degradation reaction was calculated according to Eq. (2):[18]

$$t_{1/2} = \frac{0.693}{k}. \quad (2)$$

General procedures

Construction of a calibration graph. Accurately measured volumes of the AZL standard solution were successively transferred into a series of 10-mL volumetric flasks to obtain final concentrations of 0.2 – 20.0 μg mL^{-1}; 1.0 mL of the IS solution was added, the volumes were completed to the mark with the mobile phase and solutions were mixed well. Triplicate 20 μL aliquots were chromatographed. The average peak area ratios of AZL/IS were plotted *versus* the drug concentrations (μg mL^{-1}), and the regression equation was derived.

Analysis of eye drops and nasal spray. Accurately measured volumes of eye drops and nasal spray equivalent to 1 mg AZL were transferred into 10-mL volumetric flasks, completed to the mark with the mobile phase and mixed well. The procedure for "Construction of a calibration graph" was followed. The average percentage recoveries were calculated from the regression equation.

Degradation studies

Neutral, acidic, alkaline and oxidative degradation. Aliquots of the standard AZL solution (containing 100.0 μg) were transferred into four series of screw-capped glass vials followed by 2.0 mL of distilled water, 1.0 M HCl, 0.5 M NaOH, or 9.0% w/v H_2O_2. The solutions were heated in a thermostatically controlled water bath at 80°C for acidic and oxidative degradation or at 60°C for alkaline degradation for increasing time intervals (10 – 40 min). As for neutral hydrolysis, the solution was heated in a boiling water bath for 1 h. At the specified time, the contents of the vials were cooled, and solutions under acidic and alkaline treatments were neutralized. The solutions were transferred into a series of 10-mL volumetric flasks. Then, 1.0 mL of the IS solution was added, and the volumes were completed with the mobile phase. Solutions were mixed well and triplicate 20 μL injections were made for each sample.

Photolytic degradation in the solid state and solution. Aliquots of the standard AZL solution (containing 1000.0 μg) were transferred into three glass vials and diluted to 10 mL with water, methanol or a methanol–water mixture (1:1 v/v), respectively. Approximately 200 mg of AZL powder was spread on a glass dish in a layer that was less than 2 mm in thickness. The solutions and powder were exposed to UV-lamp emitting radiation at 254 nm for 20 h at a distance of 15 cm. Samples protected from light by wrapping with aluminum foil were submitted to identical conditions and used as a control. At the specified time, 1.0 mL of each solution was accurately transferred into a 10-mL volumetric flask. Regarding the solid sample, a solution containing 100.0 μg mL^{-1} AZL was prepared in the mobile phase; then, 1.0 mL was accurately transferred into a 10-mL volumetric flask. Then, 1.0 mL of the IS solution was added and the volumes were completed with the mobile phase. Solutions were mixed well and triplicate 20 μL injections were made for each sample.

Isolation of degradation products

Acidic and alkaline degradants. After 50.0 mg of AZL was accurately weighed and dissolved in a minimum volume of acetonitrile, 20 mL of 4.0 M HCl or 4.0 M NaOH was added, and the solutions were heated under reflux at 80°C for 10 h. The solutions were neutralized and evaporated to dryness in a rotary evaporator. The obtained residues were dissolved in acetonitrile and filtered. The solution was evaporated at room temperature under a gentle nitrogen stream to obtain the degradants in a powdered form.

Oxidative degradants. After 50.0 mg of AZL was accurately weighed and dissolved in a minimum volume of acetonitrile, 20 mL of 30% H_2O_2 was added and the solution was heated under reflux at 80°C for 10 h. The solution was evaporated to dryness on a hot plate. The obtained residue was dissolved in acetonitrile and filtered. The obtained solution was fractionated using semi-preparative HPLC with the same mobile phase using a Cosmosil 40C_{18}-prep column (20 mm i.d. × 300 mm bed height), Nacalai Tesque, Inc. The two fractions corresponding to oxidative degradation products were evaporated to dryness in a rotary evaporator to obtain the two degradation products in a powdered form.

Complete degradation was ascertained by HPLC, where the peak of AZL disappeared from the chromatogram. The obtained degradation products were subjected to FAB^+ MS analysis for structural elucidation.

Results and Discussion

The first stability-indicating HPLC method for the antihistamine drug AZL was developed and validated. The method allowed for the separation of the drug from degradation products generated under ICH-recommended stress conditions[16,17] in a total run time of < 13 min. Alkaline, acidic, neutral and photolytic degradation of AZL yielded the same degradation product (DP1), while oxidative degradation yielded two degradation products (DP2 and DP3).

Method development

To achieve good separation of the analytes within a reasonable time, mobile phases of various compositions and pH values were investigated and system suitability tests (SST) were performed during method optimization.

The ratio of acetonitrile was studied over the range of 28 – 40%, v/v. Increasing the ratio of acetonitrile to 40%, v/v resulted in a decreased retention and an overlapping of AZL, DP2 and DP3. A mobile phase containing 32%, v/v acetonitrile was used allowing the separation of AZL from all degradants within a reasonable time. The pH of the mobile phase was also studied from 2.0 – 7.0. Increasing the pH of the mobile phase resulted in loss of the AZL peak sharpness and symmetry. At pH values > 3.5, DP2 and DP3 were co-eluted. Eventually, a mobile phase of pH 3.5 was chosen as being the optimum allowing for the separation of AZL and all degradation products. Studying the ionic strength of phosphate buffer (0.005 – 0.05 M) revealed no significant effect on the separation process or the retention of the analytes. Here, 0.04 M phosphate buffer was used as the aqueous phase in this study. These chromatographic parameters scarcely affected the retention of DP1.

The detection of the eluents was attempted at different wavelengths, including 210, 220, 230, and 288 nm; 210 nm was selected as the optimum detection wavelength allowing for the simultaneous detections of the drug and its degradation products with high sensitivity. The effect of the flow rate on the separation of the analytes was also investigated and a flow rate of 1.0 mL min^{-1} was optimal for good separation in a reasonable time. For choosing of a suitable IS, naftazone and flutamide were tested. Naftazone was chosen as the optimum IS, giving a symmetrical peak well separated from AZL and all of the degradation products. After this experimental study, good separation of AZL (t_R = 9.2 min) from DP1 (t_R = 1.6 min), DP2 (t_R = 10.0 min), DP3 (t_R = 11.6 min) and IS (t_R = 7.6 min) was achieved within a reasonable run time.

Method validation

The proposed method was validated according to the ICH guidelines[19] to prove its suitability for the intended purpose.

Linearity and range. The linearity was assessed by plotting the peak-area ratios of AZL/IS *versus* their respective concentrations over the range of 0.2 – 20.0 μg mL^{-1}. The high value of the correlation coefficient with a small value of the intercept indicates the good linearity of the calibration graph. Table 1 summarizes the linearity parameters for the proposed method.

Limit of quantification (LOQ) and limit of detection (LOD). LOQ and LOD were calculated according to the ICH guidelines which adopts Eqs. (3) and (4):[19]

$$\mathrm{LOQ} = 10S_a/b, \quad (3)$$

$$\mathrm{LOD} = 3.3S_a/b. \quad (4)$$

Here, S_a is the standard deviation of the intercept of the regression line and b the slope of the regression line. The results are listed in Table 1.

Accuracy. The accuracy of the proposed method was proven by recovery studies across the working concentration range. The obtained results were favorably compared with those of the comparison method[13] using the Student's *t*-test and the variance ratio F-test,[20] indicating no significant difference between the performance of the two methods regarding the accuracy and the precision, respectively (Table 2).

Precision. The intra-day precision was assessed by triplicate analysis of three different concentrations of the drug at three successive times within the same day. The inter-day precision was also evaluated by repeated analyses of three concentrations on three successive days. The small values of %RSD and %error proved the high precision of the proposed method (Table 3).

Specificity. The results of forced degradation studies revealed the ability of the proposed method to separate AZL from possible degradation products. Moreover, no interference was

Table 1 Linear regression-analysis data for the determination of AZL by the proposed method

Parameter	Result
Concentration range/μg mL^{-1}	0.2 – 20.0
Regression equation[a]	$Y = 2.48 \times 10^{-2} + 0.16X$
Correlation coefficient	0.9999
Limit of detection (LOD)/ng mL^{-1}	7.05
Limit of quantification (LOQ)/ng mL^{-1}	21.37
Standard deviation of the residuals/$S_{y/x}$	1.94×10^{-3}
Standard deviation of the intercept/S_a	3.40×10^{-4}
Standard deviation of the slope/S_b	1.10×10^{-4}
% RSD	0.76
% Error (% RSD/$\sqrt{n}$)	0.27

a. Y = peak area ratio of AZL/IS and X = concentration of AZL (μg mL^{-1}).

Table 2 Application of the proposed and comparison methods to the determination of AZL in pure and dosage forms

Matrix	Mean % Found[a] ± SD		*t*-value[d]	F-value[d]
	Proposed method	Comparison method[13]		
Pure form	100.21 ± 0.77[b]	100.11 ± 0.78[c]	0.185 (2.262)	1.037 (4.737)
Azelast® eye drops	100.01 ± 1.64[c]	99.27 ± 0.97[c]	0.676 (2.776)	2.87 (19.00)
Zalastin® metered dose nasal spray	96.77 ± 1.42[c]	95.50 ± 1.30[c]	1.150 (2.776)	1.21 (19.00)

a. Each result is the average of three independent determinations, b. n = 8, c. n = 3, d. Values between parenthesis are the tabulated *t*- and F-values at P = 0.05.[20]

Table 3 Precision data for the determination of AZL by the proposed method

Conc./ μg mL^{-1}	Intra-day precision			Inter-day precision		
	(mean % found ± SD)	%RSD	%Er	(mean % found ± SD)	%RSD	%Er
1.0	100.54 ± 1.44	1.43	0.83	99.81 ± 0.56	0.56	0.32
10.0	99.47 ± 0.84	0.84	0.49	100.94 ± 0.41	0.40	0.23
20.0	100.47 ± 1.37	1.36	0.79	100.81 ± 1.49	1.48	0.86

Table 4 Final system suitability test parameters for the proposed method

Compound	Number of theoretical plates	Resolution (DP/AZL)
AZL	7470	
DP1	1383	20.30
DP2	13961	1.91
DP3	18572	5.70

observed from excipients commonly added to pharmaceutical formulations with the peaks of interest. These results demonstrated the specificity of the proposed method.

Robustness. The method was found to be robust regarding any small variation in the pH of the mobile phase (3.5 ± 0.1), ionic strength of phosphate buffer (0.04 ± 0.001) and detection wavelength (210 ± 1 nm), as revealed by the constancy of the resolution and the peak areas. The most critical factor in the separation process is the ratio of acetonitrile, where minor variation resulted in a significant change in the retention and resolution of AZL and its oxidative degradants. Thus, this precaution should be taken in consideration when preparing the mobile phase.

Stability of the standard solution and the mobile phase. The stability of the AZL standard solution and the mobile phase was evaluated by comparing the response of aged solutions kept at room temperature with that of freshly prepared solutions. The obtained results revealed the stability of the standard solution and the mobile phase up to 7 and 2 days, respectively.

System suitability testing (SST). SST was performed as an integral part of the analytical method. The resolution and number of theoretical plates were measured as the criteria for SST (Table 4).

Comparing the analytical performance of the proposed method with reported methods revealed that it is about 2.5 – 200 times more sensitive than that from reported literature.[3–7,13–15] Though some of the reported methods exhibited higher sensitivity than the proposed method, they entail the use of a sophisticated LC-MS instrument, which requires an expert to deal with,[8–10] or only being applied to biological samples.[11,12] In addition, the proposed method is the first reported as a stability-indicating HPLC method for AZL.

Applications

Degradation behavior and stability-indicating aspects. Under alkaline and acidic hydrolysis, AZL degraded yielding the same degradation product (DP1) at 1.6 min. The degradation of AZL was found to follow first-order kinetics. The reaction rate constants (k) and the half-lives ($t_{1/2}$) were calculated using Eqs. (1) and (2), respectively (Table 5 as described earlier under "Calculation of kinetic parameters"). Mild degradation was observed upon boiling AZL with distilled water for 1h along with the appearance of DP1. The solid form of the drug showed high photo-stability, and no degradation products were observed in the chromatograms. In contrast, a methanolic solution of the drug underwent photo-degradation along with the appearance of DP1. Degradation occurred to a lesser extent in case of a methanol–water system (1:1, v/v), while no degradation was observed in the case of an aqueous solution. AZL underwent degradation under oxidative conditions with the appearance of two major degradation products (DP2 and DP3) at 10.0 and 11.6 min, respectively. Studying the degradation process kinetically revealed first-order kinetics; k and $t_{1/2}$ were calculated (Table 5). Figure 1 depicts typical chromatograms obtained from forced degradation studies of AZL.

Table 5 Kinetic parameters for the degradation of AZL under different stress conditions

Degradation condition	Reaction rate constant (k, min^{-1}) (Mean ± SD)[a]	Half life time ($t_{1/2}$, min) (Mean ± SD)[a]
Acidic degradation (1.0 M HCl, 80°C)	$2.40 \times 10^{-2} \pm 4 \times 10^{-4}$	29.88 ± 0.48
Alkaline degradation (0.5 M NaOH, 70°C)	$0.80 \times 10^{-2} \pm 1 \times 10^{-4}$	86.63 ± 1.08
Oxidative degradation (9% H_2O_2, 80°C)	$1.70 \times 10^{-2} \pm 2 \times 10^{-4}$	40.76 ± 0.48

a. Each result is the average of three independent determinations.

Mass spectrometric characterization of degradation products. The assignment of the AZL degradation products was based on comparisons of their FAB$^+$ MS with that of AZL. The mass ion peak of AZL was identified at m/z 382.22 $[M+H]^+$ and DP1 at m/z 329.17 $[M+H]^+$. The oxidative degradation products, DP2 and DP3, were found at m/z 369.4 $[M+2H]^{++}$ and 408.39 $[M+Na]^+$, respectively. Based on these results, the structures of the degradation products were elucidated. The mass spectra of AZL and degradation products as well as their structures are illustrated in Fig. 2. The structure of DP1 conforms well to our observation during the alkaline degradation study regarding the smell of strong ammonia odor, where the AZL molecule losses a nitrogen atom as NH_3, yielding DP1. The same degradation product was formed under an acidic condition as indicated by MS and HPLC studies; also the lack of ammonia odor during degradation process is attributed to its release as NH_4Cl due to the presence of HCl in the medium. Complete degradation of AZL could not be achieved under photolysis and neutral hydrolysis, since the degree of degradation was mild, even after exposure for a long time.

Pharmaceutical applications. The applicability of the developed method was verified by the determination of AZL in pharmaceutical formulations. The recoveries obtained for AZL in Azelast® eye drops were close to 100%, where those of Zalastin® nasal spray were around 96.77%. The low content of AZL in Zalastin® nasal spray was confirmed by applying a comparison method[13] (Table 2). Figure 3 illustrates the obtained chromatograms for the analyzed pharmaceutical preparations. Figure 3B depicts the chromatogram obtained for Zalastin® nasal spray, where DP1 (t_R = 1.6 min) is obvious, which corresponds to the amount of the degraded AZL.

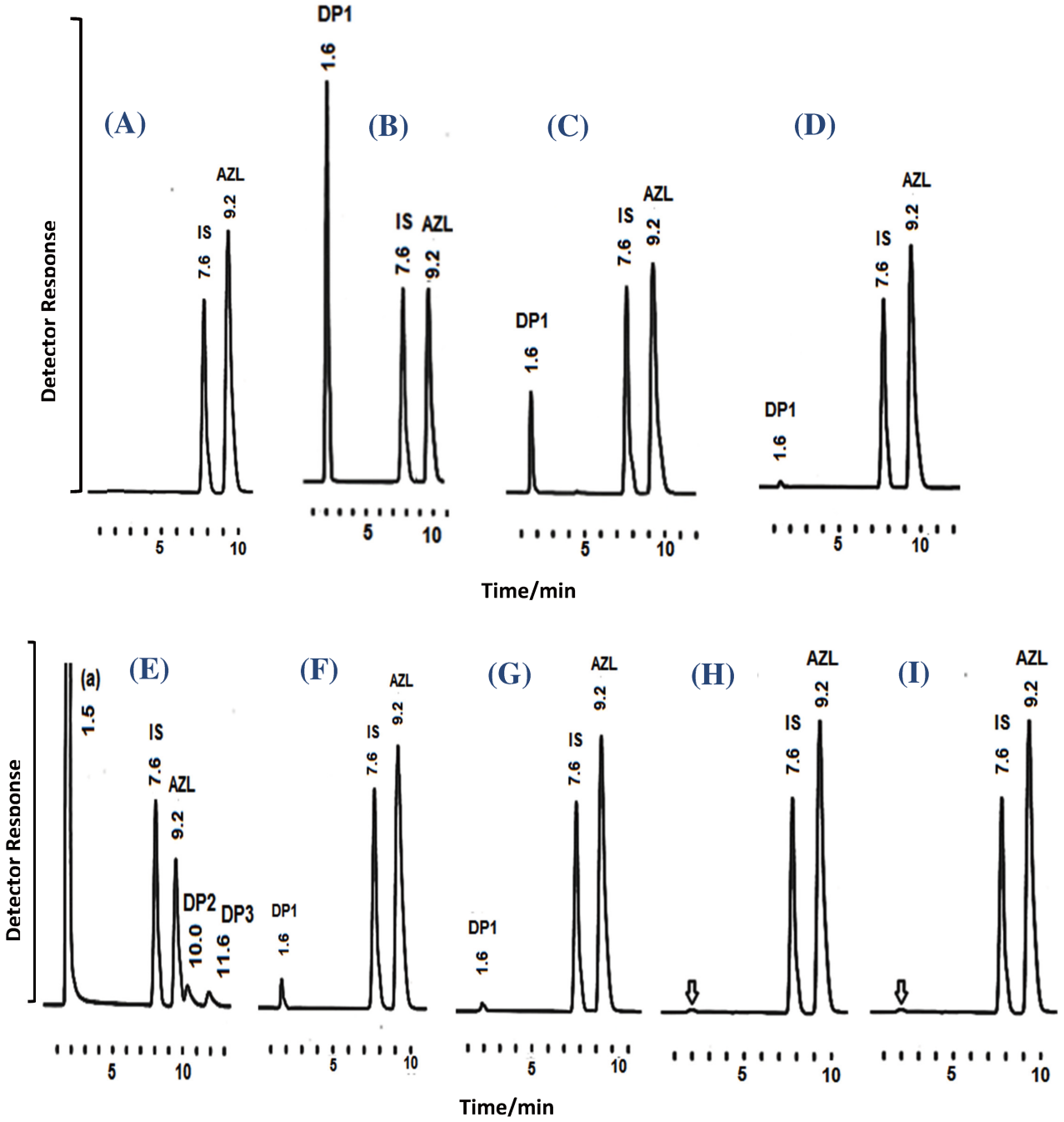

Fig. 1 Representative chromatograms illustrating the effect of different degradation conditions on AZL (10.0 µg mL^{-1}), where: (A) AZL intact drug (10.0 µg mL^{-1}), (B) Alkaline condition (0.5 M NaOH, 60°C, 30 min), (C) Acidic condition (1.0 M HCl, 80°C, 30 min), (D) Neutral conditions (water, 100°C, 60 min), (E) Oxidative condition (9.0% H_2O_2, 80°C, 20 min), (F), (G), (H), (I) UV-irradiation (254 nm for 20 h) in methanol, methanol:water 1:1, v/v, water and in solid form, respectively. Here, (DP1, DP2, DP3) are the AZL degradation products, IS is the internal standard (10.0 µg mL^{-1}) and (a) is the peroxide peak.

Conclusions

The present study represents the first stability-indicating HPLC method for AZL. A stress degradation study was conducted in order to investigate the degradation behavior of AZL under the ICH-recommended conditions; further, a kinetic investigation was performed as well with MS identification of the degradation products. The suggested method showed high sensitivity, accuracy, reproducibility and specificity, thus permitting its application in quality-control laboratories.

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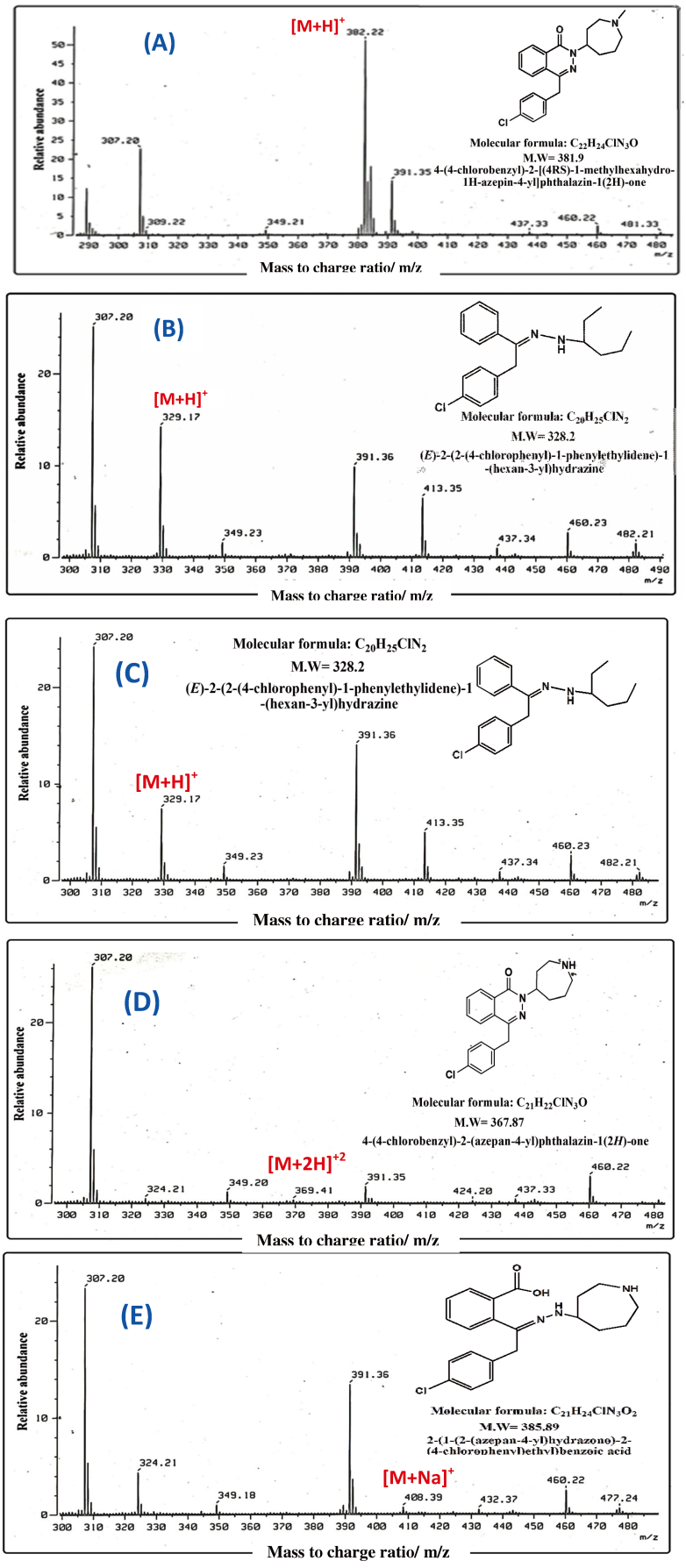

Fig. 2 Mass spectra of (A) AZL, (B) Alkaline degradation product, (C) Acidic degradation product, (D) Oxidative degradation product (DP2) and (E) Oxidative degradation product (DP3).

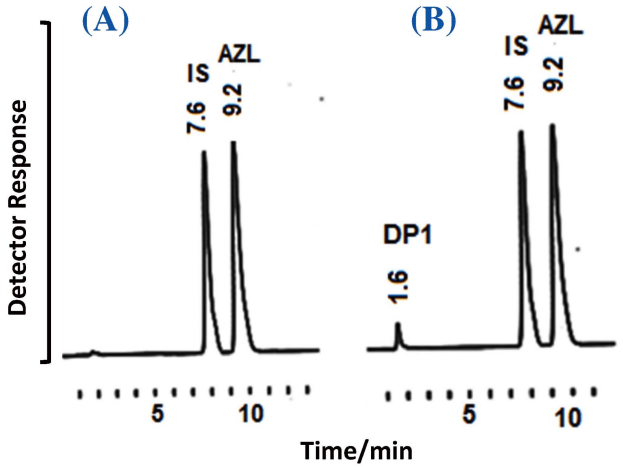

Fig. 3 Representative chromatograms for AZL (8.0 μg mL^{-1}) in (A) eye drops and (B) nasal spray. Here, DP1 is the AZL degradation product and IS is the internal standard (10.0 μg mL^{-1}).

mission in Nagasaki University, Japan.

References

1. The British Pharmacopoeia, **2012**, Her Majesty's Stationary Office, London, Electronic Version.
2. S. C. Sweetman, "*Martindale, The Complete Drug Reference*", 35th ed., **2007**, The Pharmaceutical Press, London, 568.
3. N. N. Salama, S. A. Abdel-Razeq, Sh. Abdel-Atty, and N. El-Kosy, *Bull. Fac. Pharm. (Cairo Univ.)*, **2011**, *49*, *1*, 13.
4. B. P. Rina, B. P. Bhavika, S. T. Hiren, R. P. Pinkal, K. P. Nilam, and R. P. Shirish, "*Inventi Impact: Pharm Analysis & Quality Assurance*", Vol. 2013, Article ID: "Inventi:ppaqa/904/13", available from http://www.inventi.in/Article/ppaqa/904/13.aspx (accessed 6 Apr. 2014).
5. S. A. Abdel-Razeq, M. M. Foaud, N. N. Salama, Sh. Abdel-Atty, and N. El-Kosy in "*Sensing in Electroanalysis*", Vol. 6, eds. K. Kalcher, R. Metelka, I. Švancara, and K. Vytas, **2011**, University Press Centre, Pardubice, Czech Republic, 289.
6. G. Singh and S. Rani, *Asian J. Pharm. Anal.*, **2013**, *3*, 37.
7. M. R. Elghobashy, O. M. Badran, M. Y. Salem, and K. M. Kelani, *Anal. Bioanal. Electrochem.*, **2011**, *5*, 325.
8. Y. S. Park, S. H. Kim, Y. J. Kim, S. C. Yang, M. H. Lee, L. M. Shaw, and J. S. Kang, *Int. J. Biomed. Sci.*, **2010**, *6*, 120.
9. W. Zha and L. Shum, *J. Chromatogr. B*, **2012**, *906*, 69.
10. U. Heinemann, G. Blaschke, and N. Knebel, *J. Chromatogr. B*, **2003**, *793*, 389.
11. C. N. Langevin, J. Pivonka, J. K. Wichmann, N. Kucharczyk, and R. D. Sofia, *Biomed. Chromatogr.*, **1993**, *7*, 7.
12. J. Pivonka, F. H. Segelman, C. A. Hartman, W. E. Segl, N. Kucharczyk, and R. D. Sofia, *J. Chromatogr.*, **1987**, *420*, 89.
13. B. Thangabalan and P. V. Kumar, *Int. J. Pharm. Sci. Rev. Res.*, **2012**, *17*, 62.
14. K. L. N. Rao, K. S. Babu, K. S. Raju, K. P. Reddy, K. V. Rao, and J. V. Shaik, *Int. J. Res. Pharm. Sci.*, **2010**, *1*, 473.
15. N. N. Salama, S. A. Abdel-Razeq, Sh. Abdel-Atty, and N. El-Kosy, *Br. J. Pharm. Res.*, **2014**, *4*, 79.
16. ICH, Q1A (R2), Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva, **2003**.
17. ICH, Q1B, Photostability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva, **1996**.
18. A. T. Florence and D. Attwood, "*Physicochemical Principles of Pharmacy*", 4th ed., **2006**, the Pharmaceutical Press, London, 118.
19. ICH, Q2 (R1), Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, Geneva, **2005**.
20. J. N. Miller and J. C. Miller, "*Statistics and Chemometrics for Analytical Chemistry*", 5th ed., **2005**, Pearson Education Limited, Harlow, England, 39, 107, 256.