A validated stability-indicating HPLC method for the simultaneous determination of pheniramine maleate and naphazoline hydrochloride in pharmaceutical formulations

Taomin Huang1*, Nianzu Chen1, Donglei Wang1, Yonghua Lai1 and Zhijuan Cao2*

Abstract

Background: A simple, rapid, and accurate stability-indicating reverse phase liquid chromatographic method was developed and validated for the simultaneous determination of pheniramine maleate and naphazoline hydrochloride in bulk drugs and pharmaceutical formulations.

Results: Optimum chromatographic separations among pheniramine maleate, naphazoline hydrochloride and stress-induced degradation products have been achieved within 10 minutes by using an Agilent zorbax eclipse XDB C18 column (150 mm × 4.6 mm, 5 μm) as the stationary phase with a mobile phase consisted of 10 mM phosphate buffer pH 2.8 containing 0.5% triethlamine and methanol (68:32, v/v) at a flow rate of 1 mL min⁻¹. Detection was performed at 280 nm using a diode array detector. Theoretical plates for pheniramine maleate and naphazoline hydrochloride were calculated to be 6762 and 6475, respectively. The method was validated in accordance with ICH guidelines with respect to linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation. Regression analysis showed good correlations (R² > 0.999) for pheniramine maleate in the concentration range of 150–1200 μg mL⁻¹ and naphazoline hydrochloride in 12.5–100 μg mL⁻¹. The method results in excellent separation of both the analytes and degradation products. The peak purity factor is ≥980 for both analytes after all types of stress, indicating complete separation of both analyte peaks from the stress induced degradation products.

Conclusions: Overall, the proposed stability-indicating method was suitable for routine quality control and drug analysis of pheniramine maleate and naphazoline hydrochloride in pharmaceutical formulations.

Keywords: Liquid chromatography, Method validation, Pheniramine maleate, Naphazoline hydrochloride, Degradation products

Introduction

Pheniramine maleate (pKa = 9.3), chemically known as N,N-Dimethyl-3-phenyl-3-(2-pyridyl) propylamine hydrogen maleate (C₁₆H₂₀N₂⋅C₄H₄O₄), is an antihistamine H₁ receptor antagonist by inhibiting the effect of histamine on capillary permeability, gastric secretion, and contraction of bronchiolar and gastrointestinal smooth muscle [1,2]. Recently, Kerem Karaman et al. reported that pheniramine maleate could be used as an antihistaminic for the symptomatic relief of a hypersensitivity reaction, such as the acute ocular allergic reaction and short-duration, mild to moderate, intermittent ocular allergy [3]. Naphazoline hydrochloride (pKa = 10.8), chemically designated as 2-(1-naphthylmethyl)-2-imidazoline hydrochloride (C₁₄H₁₄N₂⋅HCl), is a sympathomimetic agent with marked α-adrenergic activity and is a relatively long-lasting vasoconstrictor with a rapid action in reducing swelling when applied to the mucous membrane [4]. Figure 1 shows the structure formulae of pheniramine maleate and naphazoline hydrochloride. A novel fixed dose combination of pheniramine maleate and naphazoline hydrochloride is approved and available in
the market, indicating that it can relieve redness, burning, irritation, and dryness of the eyes caused by wind, sun, and other minor irritants.

Various techniques have been reported for the determination of pheniramine maleate and naphazoline hydrochloride, respectively. Among them, the methods for the detection of pheniramine maleate included ultra-violet spectrophotometry [5], thin-layer chromatography-densitometry [6], and high performance liquid chromatography, etc. [7,8]. Naphazoline hydrochloride in pharmaceutical formulations or biological fluids either alone or in combination with other drugs was also detected by many methods, such as spectrophotometry [9,10], gas chromatography [11], high performance liquid chromatography [12-15], capillary electrophoresis [16-18], atomic emission and atomic absorption spectrometry [19], electrochemical method [20] and luminescence method [21,22]. However, in some extent, the above-described methods are limited in either low sensitivity or specificity. Furthermore, extensive survey revealed that no stability-indicating high performance liquid chromatography (HPLC) method has been reported including major pharmacopoeias such as USP, EP, JP and BP for the simultaneous determination of these two drugs in pharmaceutical formulation. Therefore, it is necessary to develop and validate a simple and accurate stability-indicating HPLC method for simultaneous determination of both drugs and their degradation products in pharmaceutical formulations.

Results and discussion

Optimization of the Chromatographic System

There were two peaks of pheniramine maleate in the chromatography. Maleate is a dicarboxylic acid. Its ionization constant were $K_1 = 1.0 \times 10^{-2}$ and $K_2 = 5.5 \times 10^{-7}$. Pheniramine maleate was ionized pheniramine positive ion and maleate negative ion in the mobile phase. The maleate negative ion was in the front (about 1.7 min) and pheniramine positive ion was behind the maleate peak (about 3.0 min) [Figure 2]. The content of pheniramine maleate was calculated according to the peak area of pheniramine (about 3.0 min) in this study.

The main objective of our work was to develop a stability-indicating HPLC method for determination of pheniramine maleate and naphazoline hydrochloride within a short run time between 3 to 10 min and symmetry between 0.80 and 1.20. The $pK_a$ of pheniramine maleate and naphazoline hydrochloride are 9.3 and 10.8 with a UV spectral maximum response at 262 nm and 280 nm, respectively. In the commercial eye drops in the market, the content of naphazoline hydrochloride (0.25 mg·mL$^{-1}$) is far lower than that of pheniramine maleate (3 mg·mL$^{-1}$). Therefore, the wavelength of 280 nm was used for LC detection.

Both pheniramine and naphazoline hydrochloride have high carbon to heteroatom ratio and have conjugated bond. Therefore, they can be separated through C18 stationary phase based mainly on their overall hydrophobicity. Pheniramine and naphazoline hydrochloride can also be separated using phenyl-Hexyl stationary phase considering their $\pi$ electrons involving $\pi-\pi$ interactions. Finally, both drugs also contain polar functional groups. So they may be separated using cyano stationary phase.

Optimization of mobile phase, pH and stationary phase

The stationary and mobile phases play an important role on peak shape, symmetry, theoretical plates and resolution. To obtain symmetrical peaks with better resolution and no peak impurity, various chromatographic conditions was investigated and optimized for the determination of pheniramine maleate and naphazoline hydrochloride, such as mobile phase with different composition, pH and stationary phases with different packing material etc. That is, attempts were made by using three HPLC columns (Agilent zorbax eclipse XDB C18, Agilent Eclipse Plus Phenyl-Hexyl, and Dikma Platisil Cyano) with different mobile phase compositions and ratios. In all of the preceding columns, broad peaks were obtained for pheniramine and naphazoline hydrochloride by using different ratios (40:60, 50:50, 60:40, 70:30, 80:20) of methanol and water. No improvement of peak shape was obtained even when the temperature of column was enhanced to 40°C. The broad peak may be attributed to low polarity of the mobile phase. So different concentration (10 mM, 20 mM, 50 mM) of phosphate buffer was added to improve polarity of the mobile phase. The peak was narrowed but the peak symmetry was still not satisfactory. Then triethylamine (as silanol blocker) was added to the water phase, it was demonstrated that 10 mM phosphate buffer containing 0.5% v/v triethylamine was best for the improvement of peak shape. Moreover, buffer pH was found to be critical in the analytes separation and was extensively studied in method.
optimization. The effect of pH on retention was related with the ionization form of these solutes. In the attempt to investigate the effect of the mobile phase pH on the retention time and resolution of two substrates, the pH (2.8, 4.0, 5.0 and 6.0) was employed for this assay while keeping the other chromatographic parameters unchanged, i.e., Agilent zorbax eclipse XDB C18 column and the fixed mobile phase composition of 10 mM phosphate buffer containing 0.5% v/v triethlamine: methanol (68:32, v/v). As demonstrated in Table 1, a buffer pH of 2.8 was found to be optimal with narrow peak, resolution (R ≥ 3) and analysis time (t<sub>r</sub> between 3 ~ 10 min), which was then selected for the following experiments.

Following that, under the optimized mobile phase of methanol and 10 mM phosphate buffer with 0.5% v/v triethlamine (pH 2.8) (68:32, v/v), the experiments were performed on three different stationary phase. Highly symmetrical and sharp peaks of pheniramine and naphazoline hydrochloride were obtained on Agilent zorbax eclipse XDB C18 column (with better resolution, peak shapes, theoretical plates) as compared to other stationary phases (Agilent Eclipse Plus Phenyl-Hexyl, and

| Mobile phase                  | Theoretical plates (N) | Symmetry | Resolution (R) | Peak shape |
|-------------------------------|-------------------------|----------|----------------|------------|
| Methanol: Phosphate buffer, pH 2.8 (68:32) |                          |          |                |            |
| Pheniramine                   | 6762                    | 0.91     | 7.75           | +++        |
| Naphazoline hydrochloride     | 6475                    | 0.93     |                | +++        |
| Methanol: Phosphate buffer, pH 4 (68:32) |                          |          |                |            |
| Pheniramine                   | 1319                    | 0.26     | 4.13           | ___        |
| Naphazoline hydrochloride     | 4737                    | 0.43     |                | +++        |
| Methanol: Phosphate buffer, pH 5 (68:32) |                          |          |                |            |
| Pheniramine                   | 6731                    | 0.54     | 0.98           | +++        |
| Naphazoline hydrochloride     | 1325                    | 0.24     |                | ___        |
| Methanol: Phosphate buffer, pH 6 (68:32) |                          |          |                |            |
| Pheniramine                   | 6499                    | 0.54     | 2.75           | +++        |
| Naphazoline hydrochloride     | 1683                    | 0.23     |                | ___        |

Figure 2 Chromatogram for separation of pheniramine maleate and naphazoline hydrochloride in pharmaceutical formulations.
Dikma Platisil Cyano), which was used in subsequent experiments.

Method validation

The developed chromatographic method was validated using ICH guidelines [23,24]. Validation parameters included linearity, accuracy, precision, robustness, specificity, LOD and LOQ.

Linearity was verified by triplicate analysis of different concentrations. As a result, the linear regression equation was found to be \( Y = 5.0985X + 23.4917 \) \((R^2 = 0.9993, n = 6, 150–1200 \mu g \text{ mL}^{-1})\) for pheniramine and \( Y = 28.3892X + 18.8795 \) \((R^2 = 0.9999, n = 6, 12.5–100 \mu g \text{ mL}^{-1})\) for naphazoline hydrochloride, respectively. In which, \( Y \) was the dependent variable, \( X \) was independent variable, 5.0985 and 28.3892 were slopes which showed change in dependent \( Y \) variable per unit change in independent \( X \) variable; 23.4917 and 18.8795 were the \( Y \)-intercept i.e., the value of \( Y \) variable when \( X = 0 \).

Based on a signal-to-noise ratio of 3:1, LOD was found to be 0.1 and 0.02 \( \mu g \text{ mL}^{-1} \) for pheniramine maleate and naphazoline hydrochloride, respectively. LOQ with a signal-to-noise of 10:1 was found to be 0.3 \( \mu g \text{ mL}^{-1} \) for pheniramine maleate and 0.07 \( \mu g \text{ mL}^{-1} \) for naphazoline hydrochloride, respectively.

Accuracy of the developed method was determined by analyzing samples before and after the addition of known amounts of pheniramine maleate and naphazoline hydrochloride. The acceptable recovery was set as between 97.0% and 103.0% [Table 2]. The results also demonstrated that pheniramine maleate and naphazoline hydrochloride were stable in solution.

Robustness was validated by slightly varying the chromatographic conditions. In all of the deliberately varied chromatographic conditions (different flow rate, buffer composition and buffer pH), no obvious effect on the chromatographic parameters was observed [Tables 4 and 5]. Specificity was investigated by using photodiode array detection to ensure the homogeneity and evaluate purity of analyte peak. We found no interference of diluents and excipients firstly, and then the peak purity values were evaluated at different stress conditions (acid, base, oxidation, thermal and photolytic) for pheniramine maleate and naphazoline hydrochloride in formulation. As shown in Figure 3, several degradation products were detected, but had no influence on the main ingredients. The peak purity factor was more than 980 for drug product (Table 6), which further confirmed the specificity of this method.

| Table 2 Accuracy of the proposed HPLC method |
|-----------------|-----------------|-----------------|------------------|
| **Drugs**       | **Spiked**      | **Measured**    | **Accuracy (%)** |
| Pheniramine     | 150.0           | 151.2 ± 1.7     | 100.9            |
|                 | 600.0           | 612.4 ± 6.1     | 102.1            |
|                 | 1200.0          | 1187.6 ± 15.6   | 99.0             |
| Naphazoline     | 12.5            | 12.6 ± 0.1      | 101.1            |
| Hydrochloride   | 50.0            | 49.8 ± 0.6      | 99.6             |
|                 | 100.0           | 97.8 ± 0.6      | 97.8             |

| Table 3 Intra-day and Inter-day precision of the proposed HPLC method (\( n = 5 \)) |
|-----------------|---------------|-----------------|------------------|
| **Drugs**       | **Actual**    | **Intra-day**   | **Inter-day**    |
|                 | concentration | precision       | precision        |
|                 | (\( \mu g \text{ mL}^{-1} \)) | measured ± SD; RSD (%) | measured ± SD; RSD (%) |
| Pheniramine     | 150.0         | 147.8 ± 1.1; 1.0 | 147.6 ± 1.3; 1.2 |
|                 | 600.0         | 608.5 ± 2.4; 0.4 | 607.0 ± 1.3; 1.0 |
| Naphazoline     | 12.5          | 12.6 ± 0.05; 0.4 | 12.5 ± 0.1; 0.9  |
| Hydrochloride   | 50.0          | 50.3 ± 0.3; 0.5  | 50.4 ± 0.2; 0.4  |
|                 | 100.0         | 101.0 ± 0.4; 0.4 | 100.4 ± 0.5; 0.5 |

| Table 4 Robustness of pheniramine |
|-----------------|-----------------|-----------------|------------------|
| **Chromatographic condition** | **Assay %** | **\( t_r \) (min)** | **Theoretical plates** | **Symmetry** |
| Flow rate (0.9 mL min\(^{-1}\)) | 103.4 | 3.734 | 6623 | 0.89 |
| Flow rate (1 mL min\(^{-1}\)) | 101.2 | 3.013 | 6762 | 0.90 |
| Flow rate (1.1 mL min\(^{-1}\)) | 99.5 | 2.910 | 6745 | 0.91 |
| Buffer: Methanol (70:30) | 99.2 | 4.136 | 6612 | 0.87 |
| Buffer: Methanol (68:32) | 100.7 | 3.013 | 6523 | 0.88 |
| Buffer: Methanol (65:35) | 99.6 | 2.956 | 6524 | 0.86 |
| Buffer (pH 2.6) | 101.7 | 2.990 | 6456 | 0.92 |
| Buffer (pH 2.8) | 101.9 | 3.073 | 6346 | 0.85 |
| Buffer (pH 3.0) | 100.9 | 3.360 | 6678 | 0.86 |
Results of forced degradation study

All the stress conditions applied were enough to degrade two ingredients. Pheniramine was degraded up to 95.5% and naphazoline hydrochloride was degraded up to 78.5% when 5 M HCl was used. Pheniramine maleate was degraded up to 65.3% and naphazoline hydrochloride was degraded up to 44.9% when 5 M NaOH was used. Pheniramine maleate was degraded up to 84.9% and naphazoline hydrochloride was degraded up to 76.0% under oxidative stress. Pheniramine maleate was stable and naphazoline hydrochloride was degraded up to 94.3% for 240 h under thermal stress (40°C). Pheniramine maleate was stable and naphazoline hydrochloride was degraded up to 68.4% for 240 h under thermal stress (dry heat). Both pheniramine maleate and naphazoline hydrochloride were not degraded substantial under photolytic stress. From these stress studies it was thus concluded that pheniramine maleate and naphazoline hydrochloride were not stable in basic, acidic, oxidative and thermal conditions. The results of stress studies are shown in Table 6.

Comparing the results with degradation of commercial eye drops sample solution

The degradation results of commercial eye drops sample solution were illustrated in Table 7. The stability results were in conformity with that in stock solution, which demonstrated that the developed method could be used to analyze pheniramine maleate and naphazoline hydrochloride in pharmaceutical formulation.

Application of the developed method

Application of the developed method was checked by analyzing pheniramine maleate and naphazoline hydrochloride in commercially available pharmaceutical formulations. The results are provided in Table 8 which showed high percentage recoveries and low RSD (%) values for these two analytes.
Conclusions
A rapid and efficient RP-HPLC method was developed for the estimation of pheniramine maleate and naphazoline hydrochloride in pharmaceutical formulation and their degradation products. The proposed method was demonstrated to be accurate, precise, specific, sensitive, linear and robust based on method validation. Satisfactory results were obtained in separating the peaks of active pharmaceutical ingredients from the degradation products produced by forced degradation. Furthermore, the new method are cost-effective without the requirement of ion pairing and other derivatization agents, which are tend to adsorb very strongly on the stationary phase, resulting in difficulty in recovering initial column properties. Overall, the method is stability-indicating and can be used for routine analysis in quality control and any kind of stability and validation studies.

Methods
Chlorine and reagents
All chemicals were analytical grade and used as received. All solutions were prepared in Milli-Q deionized water from a Millipore water purification system (Bedford, MA, USA). Pheniramine maleate was purchased from EDQM CS30026-F67081 Strasbourg, France. Naphazoline hydrochloride with stated purify of 99.2% (lot No.100111-201104) was purchased from the National Institute for the Control of Pharmaceuticals and Biological products, Beijing, China. Pheniramine maleate and naphazoline hydrochloride eye drops (claimed to contain 3 mg mL⁻¹ pheniramine maleate and 0.25 mg mL⁻¹ naphazoline hydrochloride) were obtained from S.A. Alcon-couvreur N.V., Rijksweg 14,2870, Puurs, Belgium (lot No. 10D09L,11F09H). The eye drops contains benzalkonium chloride as preservative in sterile aqueous base. Potassium dihydrogen phosphate (KH₂PO₄) was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Triethlamine (HPLC grade) was obtained from Fisher scientific (New Jersey, USA). Phosphoric acid was obtained from Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). HPLC- grade methanol was obtained from TEDA (OH, USA). Mobile phase was filtered using 0.45 μm nylon filters from Millipore Co. (MA, USA).

Equipment and chromatographic conditions
Samples were analyzed on an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA), attached with a G1311A quaternary pump, a G1312A vacuum degasser, and a G1315B DAD detector. The detector wavelength was fixed at 280 nm and peak areas were integrated automatically using the Hewlett-Packard Chem Station software program. Other apparatus included an ultrasound generator and a SevenEasy pH meter.

Table 6 Stress testing results of pheniramine and naphazoline hydrochloride in stock solution

| Nature of stress | Storage conditions | Time (h) | Amount of pheniramine Remaining ± SD (%) | Amount of naphazoline hydrochloride Remaining ± SD (%) | Extent of decomposition |
|------------------|-------------------|----------|----------------------------------------|------------------------------------------------------|------------------------|
| 5 M HCl          | 40°C              | 24       | 95.5 ± 0.9 (PP = 999.213)               | 78.5 ± 3.8 (PP = 999.412)                              | Substantial            |
| 5 M NaOH         | 40°C              | 2        | 65.3 ± 1.4 (PP = 999.346)               | 44.9 ± 2.6 (PP = 999.015)                              | Substantial            |
| 6% H₂O₂         | 40°C              | 24       | 84.9 ± 1.6 (PP = 999.803)               | 76.0 ± 1.5 (PP = 999.711)                              | Substantial            |
| Thermal          | 40°C              | 120      | 98.2 ± 1.6 (PP = 999.872)               | 102.5 ± 1.0 (PP = 999.784)                             | None                   |
|                  | 40°C              | 240      | 99.1 ± 1.3 (PP = 999.423)               | 94.3 ± 1.2 (PP = 999.651)                              | Substantial            |
| Dry heat         | 105°C             | 7        | 99.2 ± 2.9 (PP = 999.903)               | 68.4 ± 1.9 (PP = 999.806)                              | Substantial            |
| Photolytic       | 1.2 million lux hours and 200 W h/m² | 98.2 ± 1.7 (PP = 999.312) | 98.5 ± 1.2 (PP = 999.104) | None |

n = Average of 3 determinations. PP = peak purity factor, peak purity values in the range of 980 ~ 1000 indicate a homogeneous peak.

Table 7 Stress testing results of pheniramine and naphazoline hydrochloride in commercial eye drops sample solution

| Nature of stress | Storage conditions | Time (h) | Amount of pheniramine Remaining ± SD (%) | Amount of naphazoline hydrochloride Remaining ± SD (%) | Extent of decomposition |
|------------------|-------------------|----------|----------------------------------------|------------------------------------------------------|------------------------|
| 5 M HCl          | 40°C              | 24       | 93.4 ± 0.7                              | 80.2 ± 0.4                                           | Substantial            |
| 5 M NaOH         | 40°C              | 2        | 61.3 ± 1.0                              | 48.7 ± 1.6                                           | Substantial            |
| 6% H₂O₂         | 40°C              | 24       | 87.8 ± 2.3                              | 72.1 ± 1.8                                           | Substantial            |
| Thermal          | 40°C              | 120      | 99.2 ± 1.1                              | 101.4 ± 1.4                                          | None                   |
|                  | 40°C              | 240      | 98.5 ± 1.4                              | 93.3 ± 1.8                                           | Substantial            |
| Dry heat         | 105°C             | 7        | 98.6 ± 2.4                              | 65.7 ± 2.5                                           | Substantial            |
| Photolytic       | 1.2 million lux hours and 200 W h/m² | 97.6 ± 1.9 | 98.6 ± 2.3 | None |

n = Average of 3 determinations.
(Mettler Toledo) that was equipped with a combined glass-calomel electrode. An electric-heated thermostatic water bath (DK-S28) and an oven (DGH-9203A) for thermal degradation were purchased from Shanghai Jing Hong Laboratory Instrument Co. Ltd (China). Photo stability studies were performed on a photo stability test chamber model Pharma 500-L (Weiss Technik UK Ltd., Germany). An Agilent zorbax eclipse C18 column (150 mm × 4.6 mm i.d., 5 μm) was maintained at 30°C. The mobile phase was composed of a mixture 10 mM phosphate buffer (pH 2.8) containing 0.5% triethlamine and methanol in the ratio of (68:32, v/v). The flow rate of the mobile phase was set at 1 mL min⁻¹. Measure-

| Batch no. | Compounds                | Labeled  | Found         | RSD (%) |
|-----------|--------------------------|----------|---------------|---------|
| 10009L    | Pheniramine maleate      | 45 mg 15 mL⁻¹ | 46.44 mg 5 mL⁻¹ | 0.57    |
|           | Naphazoline hydrochloride| 3.75 mg 15 mL⁻¹ | 3.98 mg 15 mL⁻¹ | 1.04    |
| 11F09H    | Pheniramine maleate      | 45 mg 15 mL⁻¹ | 46.05 mg 15 mL⁻¹ | 0.20    |
|           | Naphazoline hydrochloride| 3.75 mg 15 mL⁻¹ | 3.90 mg 15 mL⁻¹ | 1.09    |

Accuracy of the developed method was determined by standard addition method. For this purpose, known quantities of pheniramine maleate (150, 600, 1200 μg mL⁻¹) and naphazoline hydrochloride (12.5, 50, 100 μg mL⁻¹) were supplemented to the sample solution previously analyzed. Then, the experimental and true values were compared [24].

The precision was tested by intra-day and inter-day precision at three level concentrations of standard mixture for pheniramine maleate (150, 600, 1200 μg mL⁻¹) and naphazoline hydrochloride (12.5, 50, 100 μg mL⁻¹). Intra-day precision was studied on the same day (n = 5). And inter-day precision was determined by performing the same procedures on three consecutive days. Percent-

Procedure for forced degradation study
Forced degradation was carried out using different ICH [25,26] prescribed stress conditions such thermolytic, photolytic, acid, base hydrolytic and oxidative stress conditions.

Acid degradation
For this purpose, 2 mL of the standard stock solution was transferred into a 10 mL volumetric flask. And then 2 mL 5 M HCl was added into the flask, which was kept at 40°C for 24 h in water bath [24]. After completion of
the stress, the solution was cooled in room temperature and neutralized by using 5 M NaOH and the volume was completed up to the mark with mobile phase.

Alkali degradation
For this purpose, 2 mL of the standard stock solution was transferred into a 10 mL volumetric flask. Add 2 mL 5 M NaOH in the flask and keep at 40°C for 2 h in water bath. After completion of the stress, the solution was cooled in room temperature and neutralized by using 5 M HCl and diluted to the mark with mobile phase.

Oxidative degradation
For this purpose, 2 mL of the standard stock solution was transferred into 10 mL volumetric flask. Add 2 mL 6% H₂O₂ added in the flask and keep at 40°C for 24 h in water bath. After completion of the stress, the solution was cooled in room temperature and diluted to the mark with mobile phase.

Thermal degradation
Thermal degradation studies were performed at two different temperatures: 40°C in water bath and 105°C in oven (dry heat thermolysis). For thermal degradation at 40°C, 2 mL of the standard stock solution was transferred into 10 mL volumetric flask and kept at 40°C in water bath for 120 h and 240 h. After completion of the stress, the solution was cooled in room temperature and the volume was completed up to the mark with mobile phase. For dry heat thermolysis, 150 mg pheniramine maleate and 12.5 mg naphazoline hydrochloride were mixed in Petri dish at 105°C for 7 h. After completion of the stress, the powder mixture was dissolved and diluted to 50 mL with mobile phase. 2 mL of this solution was further diluted to 10 mL with mobile phase.

Photolytic degradation
Study was performed on dark control and photolytic exposed sample in a way to get the minimum exposure of 1.2 million lux hours for light and 200 W h/m² for ultraviolet region.

Degradation of commercial eye drops sample solution
The degradation of commercial eye drops sample solution was performed under the same above-mentioned stress conditions as the stock solution, including thermo-lytic, photolytic, acid, base hydrolytic and oxidative stress conditions.

Abbreviations
HPLC: High performance liquid chromatography; DE: Degradation products.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
TMH: Participate in method development and optimization, perform the experiments for forced degradation studies, collect experimental data and write the manuscript. NZC: Propose and supervise the implementation of various experiments and write the manuscript. DLW and YHL: Participate in the experiments for method validation. All authors read and approved the final manuscript.

Received: 23 September 2013 Accepted: 27 January 2014
Published: 1 February 2014

References
1. Parente G, Pazzaglia M, Vincenzi C, Tosti A: Contact dermatitis from pheniramine maleate in eye drops. Contact Dermat 1999, 40:338–338.
2. Greiner JV, Udeli IJ: A comparison of the clinical efficacy of pheniramine maleate/naphazoline hydrochloride ophthalmic solution and olopatadine hydrochloride ophthalmic solution in the conjunctival allergen challenge model. Clin Ther 2005, 27:568–577.
3. Karabacak K, Bostanci EB, Aksoy E, Ulas M, Yigit T, Erdemli MO, Ercin U, Bilgihan A, Saydam G, Akoğlu M: Effects of dexamethasone and pheniramine hydrogen maleate on stress response in patients undergoing elective laparoscopic cholecystectomy. Am J Surg 2013, 205:21–219.
4. Melouna M, Syrový T, Vrana A: The thermodynamic dissociation constants of amboxrol, antazoline, naphazoline, oxymetazoline and ranitidine by the regression analysis of spectrophotometric data. Talanta 2004, 62:511–512.
5. Raghav MS, Basaviah K, Ramesh PJ, Abdulrahman SAM, Vinay KB: Development and validation of a uv-spectrophotometric method for the determination of pheniramine maleate and its stability studies. J Appl Spectrosc 2012, 79:131–138.
6. Subramaniam SP, Das SK: Rapid identification and quantification of chlorpheniramine maleate or pheniramine maleate in pharmaceutical preparations by thin-layer chromatography-densitometry. J AOAC Int 2004, 87:1319–1322.
7. Eligazy SM, Ahmed AEHN: High-performance liquid-chromatographic determination of mepyramine maleate, pheniramine maleate and phenylpropanolamine hydrochloride in tablets and drops. Analyst 1987, 112:967–989.
8. Murata CM, Silva RA, Fernandes LP, Masi AN: Online naphazoline quality control by micellar-enhanced spectrophotometry. J Biomol Tech 2011, 26:837–875.
9. Goicoechea H, Olivier A: Chromatographic assisted simultaneous spectrophotometric determination of four-component nasal solutions with a reduced number of calibration samples. Anal Chim Acta 2002, 453:289–300.
10. Souri E, Amanlou M, Farsam H, Afshari A: A rapid derivative spectrophotometric method for simultaneous determination of naphazoline and antazoline in eye drops. Chem Pharm Bull 2006, 54:119–122.
11. Massaccesi M: Gas-chromatographic determination of some imidazolines in pharmaceutical preparations through the use of the FFAP stationary phase. Pharm Acta Helv 1967, 62:302–305.
12. Santoni G, Medica A, Grattari P, Furlanetto S, Pirzauti S: High-performance liquid-chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions. Farmaco 1994, 40:751–754.
13. Ruckmick SC, Marsh DF, Duan DT: Synthesis and identification of the primary degradation product in a commercial ophthalmic formulation using mmr, ms, and a stability-indicating hplc method for antazoline and naphazoline. J Pharm Sci 1995, 84:502–507.
14. Bauer J, Krogh S: High-performance liquid-chromatographic stability-indicating assay for naphazoline and tetrahydrozoline in ophthalmic preparations. J Pharm Sci 1983, 72:1347–1349.
15. Chochochou P, Satsinsky D, Sööki P: Fast simultaneous spectrophotometric determination of naphazoline nitrate and methylparaben by sequential injection chromatography. Talanta 2006, 70:409–413.
16. Lemus Gallego JM, Perez Arroyo J: Determination of prednisolone, naphazoline and phenylephrine local pharmaceutical preparations by micellar electrokinetic chromatography. J Sep Sci 2003, 26:947–952.
17. Marchesi AF, Willimer MK, Mantovani VE, Robles JC, Goicoechea HC: Simultaneous determination of naphazoline, diphenhydramine and...
phenylephrine in nasal solutions by capillary electrophoresis. J Pharm Biomed Anal 2003, 31:39–46.
18. Lemus Gallego JM, Perez Arroyo J: Determination of prednisolone and the most important associated compounds in ocular and cutaneous pharmaceutical preparations by micellar electrokinetic capillary chromatography. J Chromatogr B 2003, 784:39–47.
19. Khalil S: Analytical application of atomic emission and atomic absorption spectrometry for the determination of imidazoline derivatives based on formation of ion associates with sodium cobaltinitrite and potassium ferricyanide. Mikrochim Acta 1999, 130:181–184.
20. Ghoresi SM, Behpour M, Nabi M: A novel naphazoline-selective membrane sensor and its pharmaceutical applications. Sens Actuators B Chem 2006, 113:563–569.
21. Casado-Terrones S, Fernandez-Sanchez JF, Canabate Diaz B, Segura Carretero A, Fernandez-Gutierrez A: A fluorescence optosensor for analyzing naphazoline in pharmaceutical preparations-Comparison with other sensors. J Pharm Biomed Anal 2005, 38:785–789.
22. Diaz BC, Terrones SC, Carretero AS, Fernandez JM, Gutierrez AF: Comparison of three different phosphorescent methodologies in solution for the analysis of naphazoline in pharmaceutical preparations. Anal Bioanal Chem 2004, 379:30–34.
23. ICH (Q2B): Note for Guidance on Validation of Analytical Procedures: Methodology. Geneva: International conference on Harmonization, IFPMA; 1996.
24. Razzaq SN, Khan IU, Mariam I, Razzaq SS: Stability indicating HPLC method for the simultaneous determination of moxifloxacin and prednisolone in pharmaceutical formulations. Chem Cent J 2012, 6:94–103.
25. ICH (Q1A): Stability Testing of New Drug Substances and Products. Geneva: International Conference on Harmonization, Switzerland; 2003.
26. ICH (Q1B): Photostability Testing on New Drug Substances and Products. Geneva: International Conference on Harmonization, Switzerland; 1996.

doi:10.1186/1752-153X-8-7
Cite this article as: Huang et al: A validated stability-indicating HPLC method for the simultaneous determination of pheniramine maleate and naphazoline hydrochloride in pharmaceutical formulations. Chemistry Central Journal 2014 8:7.