Original Article

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A new stability indicating chiral reverse phase high performance liquid chromatography method for determination of degradation products in orphenadrine citrate

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Abstract

An enantiomeric separation of stability-indicating high performance liquid chromatographic method was developed and validated for the analysis of orphenadrine enantiomers. The degradation behaviour of orphenadrine was investigated under different stress conditions recommended by International Conference on Harmonization (ICH). Enantiomeric resolution of the drug and complete separation from its degradation products were successfully achieved on a Phenomenex® lux cellulose 1 C_{18} (250 mm × 4.6 mm i.d, 5 µm particle size) column, using UV detector at a wavelength of 241 nm, with mobile phase consisting of acetonitrile, 20mM ammonium bicarbonate at the ratio of 75:25 (v/v), and a flow rate of 1 mL/min. The drug was subjected to alkaline, acidic, neutral, oxidative and photolytic conditions in order to mimic stress conditions. The degradation products were well resolved from main peak and, proving the stability-indicating power of the method. The developed method provided linear responses within the concentration range 2 - 10 µg/mL, and regression analysis showed a correlation coefficient value (r^2) of 0.999. The HPLC method was validated as per ICH guidelines with respect to specificity, precision, linearity and robustness. Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.50 µg/mL and 2.00 µg/mL, respectively.

Keyword: Orphenadrine, Chiral, RP-HPLC, Stability-indicating method.

1. INTRODUCTION

Orphenadrine citrate is one of the anticholinergic drug that belongs to the ethanolamine category and antihistamine class. It is used to treat painful muscle spasm, as well as the treatment of Parkinson's disease. The salts of orphenadrine are available in two varieties, namely hydrochloride and citrate. The hydrochloride salt is used for the treatment of Parkinson's disease while the citrates form as a skeletal muscle relaxant. The systematic (IUPAC) name of orphenadrine citrate is (±)-N, N-Dimethyl-2-[(o-methyl-a-phenyl benzyl) oxy] ethylamine citrate and the chemical structure of orphenadrine citrate is shown in the (Figure 1) [1].

Drug orphenadrine is in the form of racemic mixture, the numerous side effects (dry mouth, dizziness, drowsiness, restlessness, insomnia, constipation, urine retention, orthostatic hypotension, and euphoria) of orphenadrine is due to d-isomer or L-isomer is not known till date, due to these reasons, we made an attempt to separate enantiomers of orphenadrine. For example chiral drug such as zopiclone is an anticholinergic chiral drug in which eszopiclone is more active and less hypnotic than the R-Zopiclone, (+)-Ketamine is more potent and less toxic than its (-)-ketamine, one isomer of dopamine, used to treat Parkinson's disease, acts on nerve cells to control tremor, while the other is toxic to nerve cells [2].

The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry. With the advent of ICH guidelines, the requirement of establishment of stability-indicating assay

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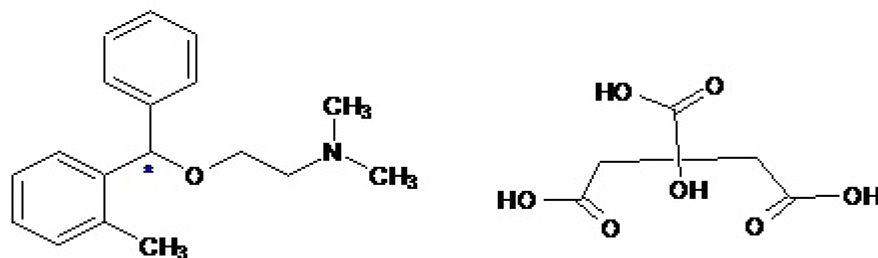

Figure 1. Chemical structure of Orphenadrine citrate

method has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry, heat, etc, and separation of drug from degradation products[3,4]. Pharmaceutical product quality is of vital importance for patient safety, the presence of impurities and potential degradation products can cause changing of chemical, pharmacological and toxicological properties of drugs having significant impact on product quality and safety. Drug stability is considered to be the secure way to ensure delivery of therapeutic values to the patients[5].

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug and degradation products generated during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the ICH guidelines[6]. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved. The ICH guidelines indicates that stress testing is designed to determine the intrinsic stability of the molecule by establishing degradation pathway in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used. ICH guidelines stability testing of new drug substance and products Q1A (R2) and (Q1B) requires that stress testing should be carried out to elucidate the substance[7]. It suggests that the degradation products that are formed under the variety of condition should include the effect of temperature, appropriate oxidation, photolysis and susceptibility[8, 9].

Similar to stability- indicating studies, enantiomeric separation of analytical techniques are important in order to determine the pharmacological and toxicological properties of chiral drugs since there may be significant variability in clinical responses between enantiomers in the same individual. HPLC is one of the well established analytical techniques commonly employed in conducting stability studies in chiral separation and also gained popularity due to its high resolution capacity, speed, sensitivity and specificity. Separation of enantiomers can be achieved using either chiral stationary phases or chiral additives to the mobile phase. The most common HPLC approach for resolving enantiomers involves the use of chiral stationary phases (CSPs)[10]. Earlier publications have described a HPLC methodology useful for the estimation of enantiomers in the presence of degradation products formed during forced degradation studies [11, 12].

None of the reported analytical methods described stability-indicating method for the chiral determination of orphenadrine in presence of its degradation products. The active or toxic forms of enantiomers were not known till date. The aim our present study is to develop and validate stability indicating chiral RP-HPLC method for enantiomeric separation of racemic orphenadrine.

2. MATERIALS AND METHODS

2.1 Solvents and chemicals

Racemic orphenadrine citrate standard was obtained from Harika drugs private limited, Hyderabad, Andhra Pradesh, India. Commercially available orphenadrine tablet was purchased commercially from the local market, Udhagamandalam, Tamilnadu, India. Chromatographic solvents such as methanol, acetonitrile and isopropyl alcohol (HPLC grade), ammonium bicarbonate, diethyl amine, triethylamine and trifluroacetic acid were obtained from S.D. Fine chemicals and water HPLC grade obtained from Milli-Q RO system was used.

2.2 Method development

Chromatographic separation and quantitative determination were performed by using a high performance liquid chromatographic system, from Shimadzu (Kyoto, Japan) equipped with LC-10 AT-VP solvent data station delivery system, an SPD M10 A UV detector, LC-2010 an HT auto sampler with loop volume of 100 µL and the class VP data station was used. The spectrum of orphenadrine was determined by using Shimadzu 1700 (E) spectrophotometer.

A phenomenex® lux cellulose 1 C_{18} (250 mm x 4.6 mm; 5 µm particle size) column was used for the enantiomeric separation of orphenadrine. The mobile phase consisted of acetonitrile, 20 mM ammonium bicarbonate in the ratio of (75: 25 v/v). The total study was performed at a flow rate of 1.0 mL/min with detection wavelength at 241 nm.

2.3 Preparation of ammonium bicarbonate buffer

Accurately weighed quantity of 1.581g of ammonium bicarbonate and transferred into a 1000 mL volumetric flask. About 900 mL of Millipore water was added and degassed by subjecting to sonication for 10 min and final volume was made with water. The buffer solution was filtered through 0.22 µm membrane filter before use.

2.4 Preparation of mobile phase:

Mobile phase was prepared by mixing 75 volumes of acetonitrile and 25 volumes of ammonium bicarbonate. The mobile phase was ultrasonicated, filtered through 0.22 µm membrane filter, and degassed.

2.5 Preparation of Stock and Standard solution

The standard racemic stock solution of orphenadrine citrate was prepared by dissolving 10 mg of drug in 10 mL volumetric flask with acetonitrile to obtain a concentration of 1 mg/mL and stored in air tight container. Furthermore, the working standard solutions were prepared by diluting with mobile phase to obtain a concentration of 10 µg/mL. The standard solution was prepared and directly injected into HPLC system (Figure 2).

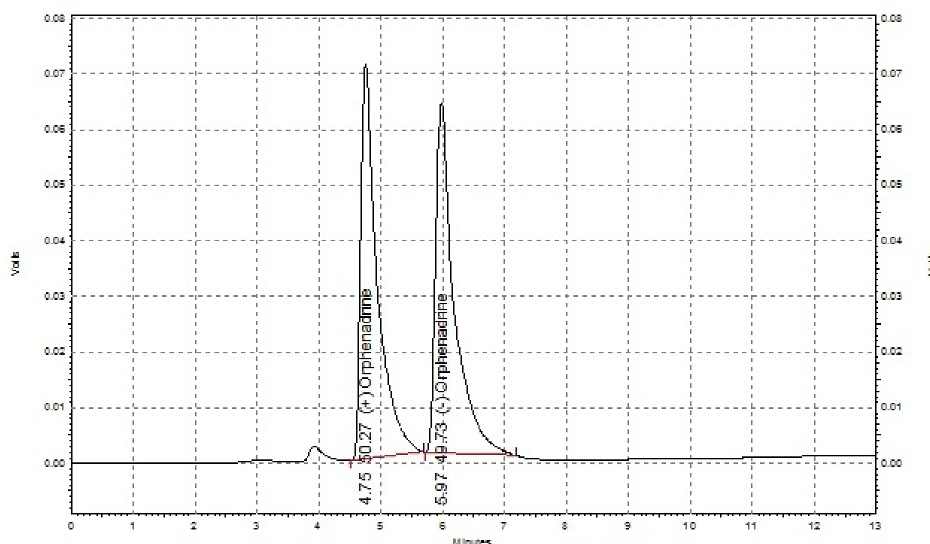

Figure 2. HPLC chromatogram of racemic (±) orphenadrine standard

2.6 Forced degradation studies

The forced degradation studies were performed to evaluate the stability of the orphenadrine method. The study was performed by using the conditions of hydrolysis, neutral, oxidation and photolysis, as per ICH guidelines. Under a particular stress condition, the drug molecule may generate different degradation products. The degradation products generated in the stressed samples are generally termed as potential degradation products which might be formed under relevant storage conditions. The hydrolytic degradation of a newly proposed drug in acidic and alkaline conditions can be studied by room temperature the same in HCl / NaOH. Furthermore, to examine the degradation by oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3-30%. The degradation studies carried out in the present work are described below.

2.7 Alkali degradation

1 mL of 1000 µg orphenadrine sample was taken into 10 mL volumetric flask, and diluted with 0.1N sodium hydroxide solution, contents were mixed well. 1 mL of this solution was taken in 10 mL volumetric flask and neutralized with 1 mL of 0.1N hydrochloric acid and diluted to 10 mL with mobile phase. These solutions were injected in HPLC system and chromatograms were recorded to assess the stability of the sample.

2.8 Acid degradation

1 mL of 1000 µg orphenadrine sample was taken into 10 mL volumetric flask, and diluted with 0.1N hydrochloric acid solution, contents were mixed well. 1 mL of this solution was taken in 10 mL volumetric flask and neutralized with 1 mL of 0.1N sodium hydroxide and diluted to 10 mL with mobile phase. These solutions were injected in HPLC system and chromatograms recorded to assess the stability of the sample.

2.9 Neutral degradation

1 mL of 1000 µg orphenadrine sample was taken into 10 ml volumetric flask, and diluted with Milli Q water, contents were mixed well. 1 mL of this solution was taken in 10 ml volumetric flask and diluted to 10 mL with mobile phase. These solutions were injected and the chromatograms recorded to assess the stability of the sample.

2.10 Oxidative degradation

1 mL of 1000 µg orphenadrine sample was taken into 10 mL volumetric flask, and diluted with 3% hydrogen peroxide, contents were mixed well. 1 mL of this solution was taken in 10 mL volumetric flask and diluted to 10 mL with mobile phase. These solutions were injected and the chromatograms recorded to assess the stability of the sample.

2.11 Photo Stability studies

1 mL of 1000 µg orphenadrine sample was taken into 10 mL volumetric flask, and diluted with mobile phase and kept in UV chamber for 24 hrs. 1 ml of this solution was taken in 10 mL volumetric flask and diluted to 10 mL with mobile phase. These solutions were injected and the chromatograms recorded to assess the stability of the sample.

3. RESULTS AND DISCUSSION

3.1 Optimization of chromatographic conditions

To develop a rugged and suitable liquid chromatographic method for the separation of orphenadrine enantiomers, different mobile phases and stationary phases were employed. The preliminary investigations were directed towards the effect of various factors on the system. The factors assessed include, the type of column and the composition of mobile phase. The following parameters, such as solubility, detection of wavelength, mobile phase buffers, mobile phase proportions, stationary phase, flow rate, column oven temperature were carefully evaluated. The optimized conditions were finally selected based on the criteria of peak properties such as resolution factor was found to be 1.4, peak asymmetry factor was found to be 1.05 for (+) Orphenadrine and 1.15 for (-) Orphenadrine

and column theoretical plate number was found to be 50598 for (+) Orphenadrine and 71111 for (-) Orphenadrine respectively.

Orphenadrine standard was dissolved in acetonitrile and a concentration of 10 µg/mL was prepared. A UV spectrum was recorded by scanning the racemic standard solution in the range of 200 nm to 400 nm. From the UV spectrum, wavelength of 241 nm was selected at which orphenadrine showed maximum absorbance. Various solvents such as acetonitrile: buffer, methanol: buffer, with different ratios, ammonium acetate buffer, ammonium formate buffer, potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and ammonium bicarbonate buffers were tested. Different acids like formic acid, acetic acid, orthophosphoric acid were used for pH adjustment of different buffers and also tried with acidic and basic peak modifiers such as trifluroacetic acid diethyl amine and triethylamine to improve the peak shape and resolution of enantiomers.

The optimum condition with methanol: ammonium bicarbonate at the ratio of 75:25 v/v were tried, a broad peak shape was observed with more tailing, to overcome this we have replaced acetonitrile in place of methanol and the remaining conditions are same, a good resolution occurred between the enantiomers and peak tailing has been reduced compared to methanol. The above conditions were tried with different solvents, pH adjustment, ratios and flow rate respectively.

The method development was finally optimized with the following conditions: The mobile phase consisting of acetonitrile, 20mM ammonium bicarbonate in the ratio of 75:25 (v/v) and Phenomenex® Lux Cellulose 1 C_{18} (250 mm x 4.6 mm i.d, 5 µm particle sizes), stationary phase. The analysis was carried out in an isocratic elution mode using a flow rate of 1.0 mL/min, injection volume of 20 µL at room temperature, and the detection of analyte was recorded at 241 nm. The mobile phase solvents were filtered through 0.22 µm membrane filter before delivering into the HPLC system. The chromatogram was recorded using Class VP software.

3.2 Method validation

The calibration curves were linear in the range of 2 – 10 µg/mL with a correlation coefficient (r^2) of 0.999 for both enantiomers. A typical calibration curve has the regression equation of y = 24920x + 1450 for (+) Orphenadrine and y = 24695x + 520.3 for (-) Orphenadrine. The limit of detection (LOD) and the limit of quantification (LOQ) for each enantiomer were 0.50 µg/mL and 2.00 µg/mL, respectively. The precision of the method was demonstrated by reproducibility studies. The mean, standard deviation and % RSD were calculated. The % RSD values were found to be less than 2% revealed that, the method were precise. Accuracy was calculated in terms of percentage recovery an analysis of the results showed that the percentage recovery value was found to be 100.85 % and 99.15 %, thus establishing that the developed method is accurate and reliable. Robustness of the method was determined by making slight changes in the chromatographic conditions such as flow rate, wavelength of detection and mobile phase ratio etc. It was observed that there were no marked changes in the chromatograms, and the results remained within the specification limit which demonstrated that the developed method was robust. System suitability parameters such as theoretical plates (N), resolution factor (R_S) and peak asymmetry factor (A_S) were calculated for the racemic standard solutions. The results validation parameters were summarized in (Table 1).

Table 1. Summary of validation parameters

S. No	Parameters	(+) Orphenadrine	(-) Orphenadrine
1	Linearity range	2 - 10 µg/mL	2 – 10 µg/mL
2	Régression équation	Y = 24920x + 1450	Y = 24695x + 520.3
3	Correlation coefficient	0.999	0.999
4	LOD (µg/mL)	0.50	0.50
5	LOQ (µg/mL)	2.00	2.00

3.3 Forced degradation studies

The amount of drug decomposed at various stress conditions are summarised in (Table 2) and (Figure 3). In basic degradation studies, it was observed that the orphenadrine enantiomers were found to show 7.50 % and 12.12 % of degradation at room temperature and no degradant peak was observed in the HPLC chromatogram (Figure 4). In acidic degradation it was observed that the orphenadrine enantiomers were found to be stable, around 8.18 % and 10.65 % of the analyte were degraded through 24 hrs and the main analyte were eluted at 4.74 and 5.97 min. Orphenadrine enantiomers were found to be stable under neutral condition up to 24 hrs. The drug enantiomers were completely degraded by using 30 % H_2O_2 at 0 hr and by using 3% H_2O_2 the drug was degraded up to 92.05 % and 93.34 % at 24 hrs for (+) and (-) orphenadrine (Figure 5). Moreover, slightly degradation was observed on photo-degradation around 12.50 % and 13.52 % were degraded through 24 hrs respectively. The drug was degraded more in oxidation condition due to the chemical reaction with tertiary amino group. The drug was found to be stable and there were no peak was observed in other degradation conditions such as hydrolysis, neutral and photolysis. From the present work it was suggested that the drug orphenadrine is stable under varied chemical environments and is not liable to undergo any chemical changes during formulation or shelf life.

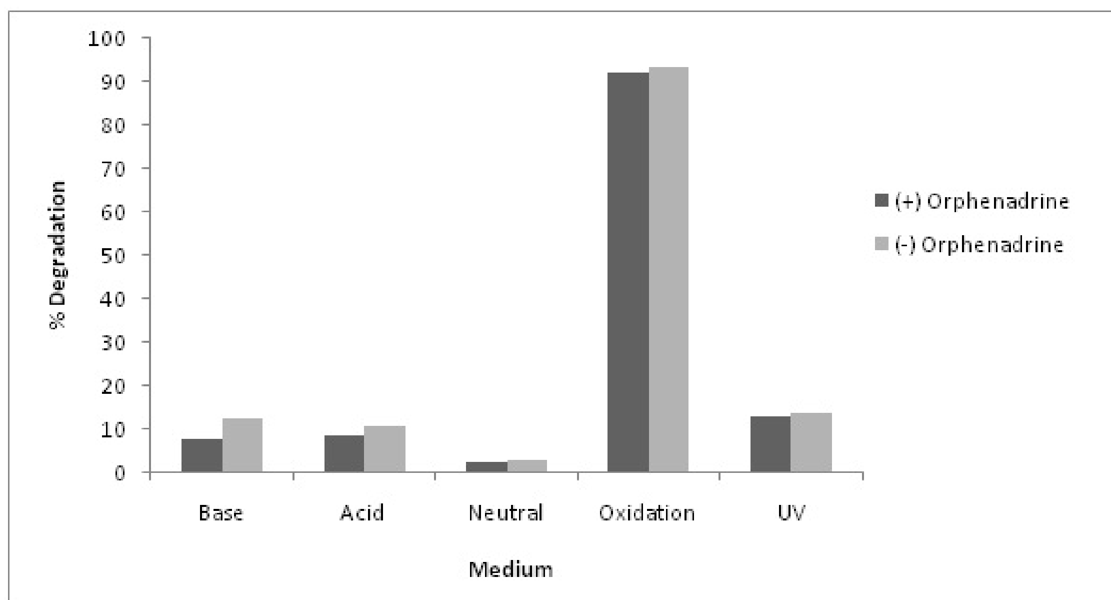

Figure 3. Bar pictorial representation of (±) Orphenadrine enantiomers

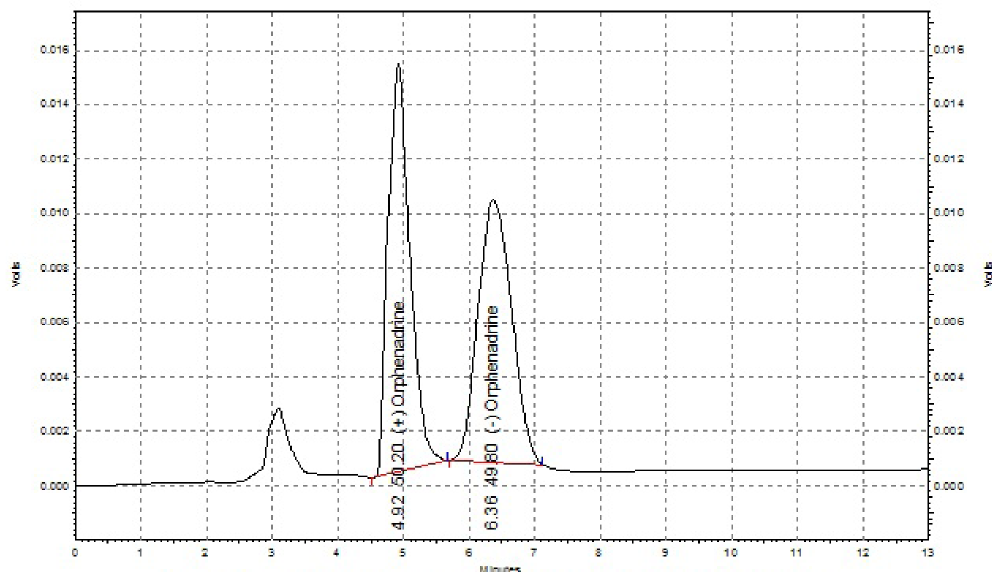

Figure 4. Typical HPLC chromatogram of basic degradation samples of orphrnadrine enantiomers with 0.1 N NaoH at 24 hrs.

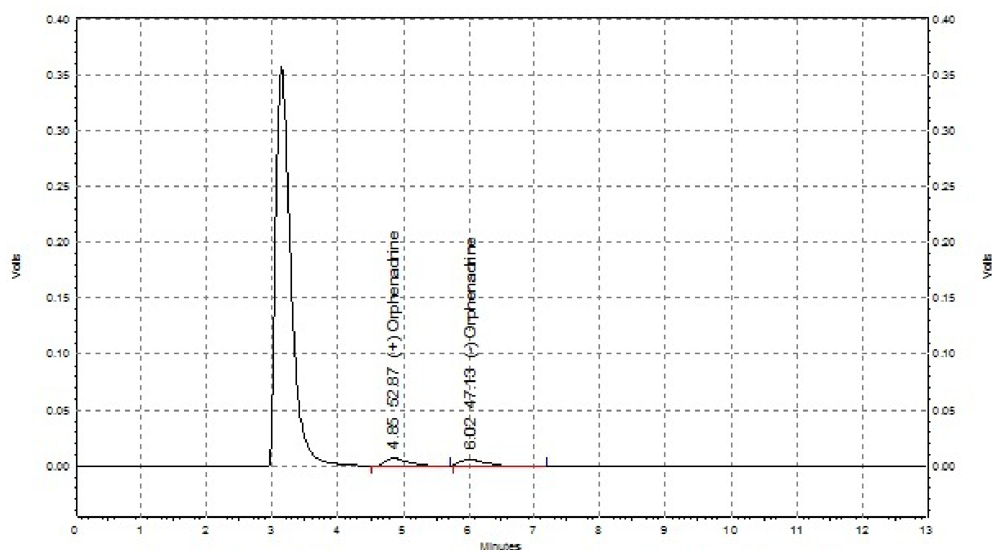

Figure 5. Typical HPLC chromatogram of oxidation condition of (±) orphrnadrine with 3% H_2O_2 at 24 hrs

4. CONCLUSION

A highly specific stability-indicating chiral HPLC method was developed for the quantification of orphenadrine enantiomers in presence of their degradation products. The enantioseparation was carried out by using cellulose based chiral column. The total run time for the developed method is 13 min. The method provides good sensitivity and excellent precision and reproducibility. The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of samples of orphenadrine in bulk drugs.

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6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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