

Stability-Indicating Reversed Phase-HPLC Method Development and Validation for Estimation of Phenylephrine in Bulk and Tablet

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ABSTRACT

Background: This study is aimed to develop a validated stability-indicating method of a nasal decongestant phenylephrine hydrochloride in bulk and tablet. **Materials and Methods:** A sensitive, accurate, and specific reversed-phase stability indicating HPLC method was developed and validated by following ICH guidelines, for the estimation of Phenylephrine Hydrochloride (PHE) in bulk and tablet. On Luna® 5µm C₁₈ column (250 × 4.6mm), the isocratic separation was achieved using mobile phase of 5mM ammonium acetate (pH 4.7): methanol (80:20; v/v) with a flow rate of 1 mL/min and a column temperature at 30°C. The proposed method was able to produce good separation of the drug and its degradation products with sharp peaks. The quantification was done at 272 nm by photodiode array detection. **Conclusion:** It was discovered that the phenylephrine hydrochloride was resilient to photolytic and thermal degradation, but degraded under acid, base, and oxidative stress conditions. The developed method was found to be linear, robust, and accurate and can be successfully applied for identification, quantitative determination, and monitoring of the stability of phenylephrine in bulk and tablet dosage forms.

Keywords: Chromatogram, High performance liquid chromatography, Decongestant, Degradation, ICH guidelines.

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INTRODUCTION

The safety of a drug and its effectiveness are greatly impacted by its chemical stability. Stability-indicating methods are quantitative analytical procedures that accurately measure the active constituent by distinguishing them from its degradants.¹ Phenylephrine hydrochloride (PHE) chemically is 3-[(1R)-1-hydroxy-2-(methylamino)ethyl]phenol hydrochloride (Figure 1), which is an effective nasal decongestant.^{2,3} It appears as odourless bitter tasting crystals which are white and are freely soluble in water and alcohol. Legerlotz was the first to prepare phenylephrine hydrochloride by the hydrogenation of m-hydroxy- ω-methylamino-acetophenone in presence of colloidal palladium.⁴

HPLC is a versatile and reproducible chromatographic technique for the resolution of a drug from its degradation products and

their simultaneous estimation. Review of literature revealed several methods for PHE estimation in combination with some other drugs, including UV-spectrophotometric method.⁵⁻⁷ Purity assessment of ebastine and PHE by Ultra-Performance Liquid Chromatography (UPLC),⁸ assay of PHE and diphenhydramine oral solutions using Ultra-High-Performance Liquid Chromatographic (UHPLC),⁹ simultaneous estimation of ascorbic acid, PHE, paracetamol and levocetirizine hydrochloride by RP-HPLC.¹⁰ HPLC-diode array detection technique for estimating phenylephrine hydrochloride, dimetindene maleate, and benzalkonium chloride simultaneously,¹¹ estimation of cetirizine along with PHE in tablet by RP-HPLC method,¹² PHE with acetaminophen, dextromethorphan, doxylamine, guaifenesin, caffeine and aspirin in various cold and cough formulations by RP-HPLC,¹³ cyclopentolate hydrochloride and PHE determination in presence of their potential degradation by HPLC,¹⁴ simultaneous determination and impurity-profiling of ebastine and phenylephrine hydrochloride by HPTLC (High-Performance Thin-Layer Chromatography),¹⁵ analysis of ketorolac tromethamine, PHE and chlorpheniramine maleate in ternary mixture using HPTLC¹⁶ and HPTLC determination of paracetamol, PHE, caffeine and chlorpheniramine in the tablet



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have been reported in the literature.² To the best of our knowledge, a stability-indicating HPLC method for PHE alone, in bulk and tablet dosage form has not yet been reported, therefore it was thought worthwhile to design a specific and precise RP-HPLC stability-indicating method for estimating PHE in bulk and tablet according to guidelines of International Conference on Harmonization (ICH) Q1A (Stability Testing of New Drug Substances and Products) and ICH Q1B (Photostability Testing of New Drug Substances and New Drug Products).

MATERIALS AND METHODS

Instrumentation

The reverse-phase chromatographic system consisting of Prominence LC-2030C 3D Plus (Shimadzu, Kyoto, Japan) equipped with a PDA (photodiode array) detector and an autosampler. Luna[®] 5 μ m C₁₈ column (250 \times 4.6mm) with 30°C column temperature and 5mM ammonium acetate (pH 4.7): methanol (80:20; v/v) as mobile phase were opted for estimating PHE in isocratic separation mode. The detection at 272 nm was fixed, and flow rate was maintained at 1mL/min. Processing and acquisition of chromatographic data were performed using Lab Solution Software (Shimadzu).

The study also used other instruments like, UV double-beam spectrophotometer: UV-1800 (Shimadzu), digital analytical balance: AUX 220 (Shimadzu, Kyoto, Japan), melting point apparatus: VMP-DS (Veego Instruments, Mumbai, India), and hot air oven: NOVA Instruments (Ahmedabad, India).

Statistical analysis

The statistical analysis was conducted using Microsoft Excel 2010 and GraphPad Prism version 4.0 software (GraphPad Software Inc., La Jolla, CA, USA).

Solvents and reagents

PHE was obtained as a gift sample from Malladi Drugs and Pharmaceuticals Ltd., Chennai, India. Solvin tablets (10 mg) of IPCA Laboratories Ltd., were purchased from a local pharmacy store. Analytical grade solvents and reagents viz methanol, ammonium acetate, and acetic acid were purchased from Merck-Millipore, India.

Preparation of standard solution

PHE (10 mg) was accurately weighed and dissolved in 10 mL methanol to get concentration of 1000 μ g/mL. Serial dilutions were performed from the stock solution using methanol to get concentration range between 20-100 μ g/mL.

Preparation of sample solution of PHE

The Solvin tablets (20) were weighed and grounded to a fine powder. A quantity equivalent to 10 mg of PHE was weighed,

dissolved in 10 mL methanol, and subjected to sonication for 15 min followed by 5 min centrifugation at a speed of 2000 rpm. The solution was filtered using a 0.45 μ m syringe filter, and the supernatant was subjected to analysis.

Preparation of 5mM ammonium acetate solution

A 5mM ammonium acetate solution was prepared by dissolving 385.4 g in 1000 mL water. A sufficient volume of acetic acid was added to bring the pH to 4.7 followed by filtration using a 0.45 μ m membrane filter.

Method Validation

For method validation ICH guidelines Q2R (R¹) were adopted.

Linearity and range

Linearity was studied at five concentrations of PHE (20-100 μ g/mL) in triplicates. The working standards were prepared by diluting the standard solution to obtain concentrations in above mentioned range. Calibration plot was constructed between peak area and concentrations to calculate the standard deviation (σ), coefficient of correlation (r^2), and residual analysis.

Sensitivity

The Detection Limit (LOD) and Quantitation Limit (LOQ) were evaluated to establish the sensitivity of the method and was calculated using the following equations as per ICH guidelines.

$$\text{LOD} = 3.3 \times \text{Standard deviation of the response/Slope of the calibration curve}$$

$$\text{LOQ} = 10 \times \text{Standard deviation of the response/Slope of the calibration curve}$$

Standard deviation values were computed using residual values between observed and predicted values shown in regression analysis.

Accuracy

Recovery studies were performed by addition of 20, 40, and 60 μ g/mL of a standard solution of PHE to the fixed concentration (40 μ g/mL) of the sample solution. Each solution was injected in triplicates and recovery was calculated by measuring the area of the peak and fitting the values into the regression equation of the calibration curve. The average % recovery was then calculated.

Precision

Three PHE concentrations (20 μ g/mL, 40 μ g/mL, 60 μ g/mL) were injected in triplicates to calculate % relative standard deviation (%RSD). Intra-day precision was determined by injecting the concentrations in triplicates on the same day, while inter-day precision was repeated on three consecutive days.

Table 1: Final chromatographic conditions.

Chromatographic parameters	Optimized conditions
Stationary Phase	Luna® 5µm C ₁₈ column (250×4.6mm)
Mobile Phase	5mM ammonium acetate (pH 4.7): methanol (80:20; v/v)
Flow rate	1mL/min
Column temperature	30°C
Injection volume	20 µL
Detection wavelength	272 nm

Table 2: Summary of method validation parameters.

Parameters	Result
Concentration range (µg/mL) <i>n</i> =5	20-100
Best fit values	
y- intercept	-13780
Slope	3478
Goodness of fit	
Correlation coefficient(<i>r</i> ²)	0.9965
Sensitivity	
LOD (µg/mL)	6.1658
LOQ (µg/mL)	18.6843
Precision	
Intra-day (repeatability) <i>n</i> =3 %RSD	0.560
Inter-day (reproducibility) <i>n</i> =3 %RSD	0.560284 / 0.548458 / 0.586016
% Recovery	99.6739
Specificity	Specific

Robustness

It was studied by evaluating small but deliberate variations which effect in the chromatographic conditions. Small variations were made in flow rate (1mL/min ± 0.1mL/min), in wavelength detection (272 ± 2 nm) and column temperature (30°C ± 2°C). The results were statistically compared.

Assay

Twenty tablets of Solvin were triturated into a fine powder, an amount equivalent to 10 mg was taken and dissolved in methanol. The solution was sonicated for 15 min and filtered using a 0.45 µm syringe filter before analysis.

Degradation studies

The studies were performed according to guidelines given in ICH Q1A and ICH Q1B.

Acid-induced degradation

In this study 10 mg of PHE was dissolved in 10 mL 0.1N methanolic hydrochloric acid solution, it was refluxed at 60°C for 1 hr in the dark to avoid any interference of light.

Base-induced degradation

In basic degradation, PHE (10 mg) was dissolved in 10 mL 0.1N methanolic sodium hydroxide solution and was refluxed at 60°C for 1 hr in the dark.

Oxidative degradation

A drug concentration of 1mg/mL was prepared using methanol, the solution was further treated with 10 mL H₂O₂ (6% v/v) for 1 hr in dark.

Photodegradation

The drug was kept for 8 hr (each day up to six days) in direct sunlight, for photochemical degradation.

Thermal degradation

In the thermal degradation study, PHE (10 mg) was kept at 60°C for 4 hr in an oven, and then solution was made using 10 mL methanol to get the concentration of 1 mg/mL.

RESULTS

Method-development and validation

The physicochemical properties of PHE were considered while optimizing the conditions of method development. Different columns, mobile phases, and flow rates ranging from 0.4–1.0 mL/min were tried. Finally, a column of C18 column having dimension 250 × 4.6 mm, 5 µm, isocratic elution mode with a mobile phase of 5mM ammonium acetate (pH 4.7): methanol (80:20; v/v) at 30°C and flow rate of 1 mL/min were chosen to optimize the method of analysis. The UV spectrum showed an absorption max at 272 nm (Table 1). The total run time was reduced to 5 min as the retention time of PHE was obtained at 2.6 min (Figure 2A).

Linearity

A linear relationship was exhibited in the calibration curve between the area of peak and concentration ranging from 20-100 µg/mL (five data points) in triplicates. Linear function gave the best correlation (0.996 ± 0.002) (Table 2). Furthermore, the residual plot analysis also ensured the random distribution of residuals around the zero value (Figure 3A and 3B).

LOD and LOQ

The detection limit was found to be 6.1658 µg/mL and the quantitation limit was found 18.6843 µg/mL respectively (Table 2).

Table 3: Accuracy studies of PHE.

Amount of sample taken ($\mu\text{g/mL}$)	Amount of standard added ($\mu\text{g/mL}$)	Percentage of standard added	% Recovery	% Average recovery
40	20	50	99.033	99.6739
40	40	100	99.635	
40	60	150	100.352	

Table 4: Robustness of method.

Parameters	Changes in parameter	Mean peak area	% RSD
Flow rate ($\pm 0.1\text{mL/min}$)	0.9	272092	0.53
	1	271261	
	1.1	272101	
Wavelength detection ($\pm 2\text{nm}$)	270	271215	0.591
	272	272001	
	274	272110	
Column temperature ($\pm 2^\circ\text{C}$)	28	271261	0.604
	30	271215	
	32	272001	

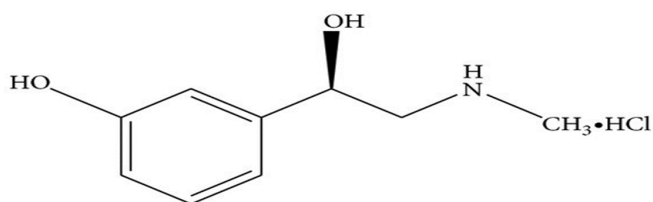
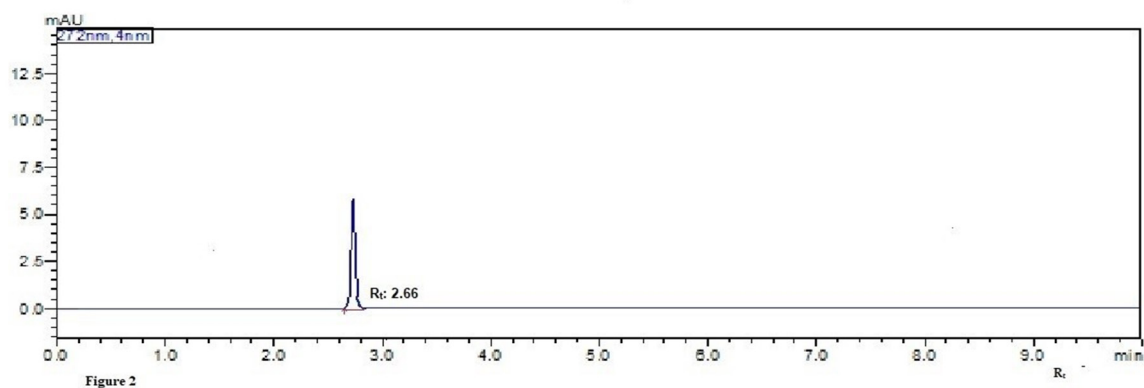
**Figure 1:** Structure of PHE.**Figure 2:** Chromatogram of PHE.

Table 5: Stress degradation studies of PHE.

Stress type	Stress condition	R _t of PHE	R _t of degradation product	% Degradation
Acid hydrolysis	0.1N HCl, 1 hr at 60°C	2.66	3.58	21.92
Alkali hydrolysis	1N NaOH, 1 hr at 60°C	2.7	3.81	19.39
Oxidative degradation	6% v/v H ₂ O ₂ , 1 hr (room temperature)	2.7	3.1	7.14
Thermal degradation	4 hr at 60°C	No degradation product was detected		
Photochemical degradation	Sunlight exposure for 48 hr	No degradation product was detected		

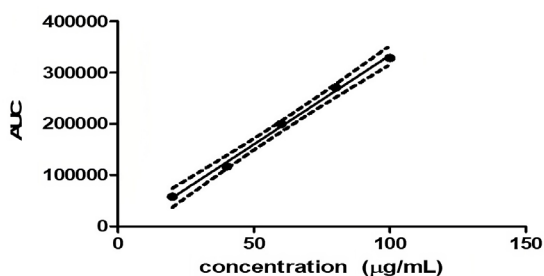


Figure 3A

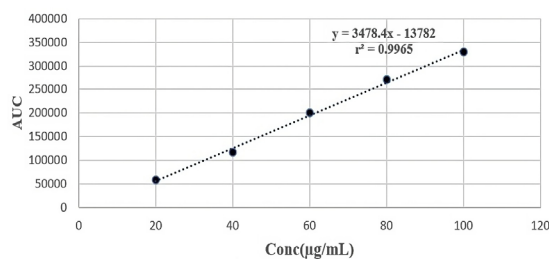


Figure 3B

Figure 3: (A) Residual plot of PHE (B) Linearity graph of PHE.

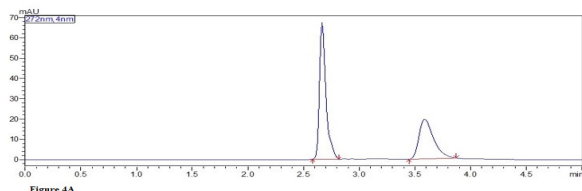


Figure 4A

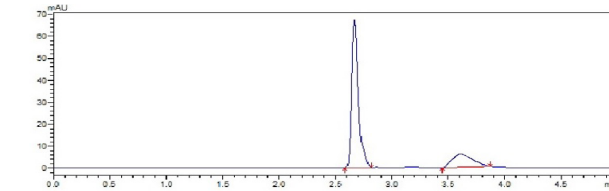


Figure 4B

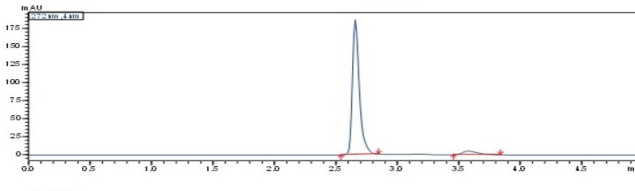


Figure 4C

Figure 4: Degradation of PHE (A) Acid degradation: peak 1 (PHE R_t: 2.66), peak 2 (degradant R_t: 3.58); (B): Alkaline degradation: peak 1 (ALA R_t: 2.7), peak 3 (degradant R_t: 3.81); (C): Oxidative degradation: peak 1 (ALA R_t: 2.7), peak 4 (degradant R_t: 3.7).

Accuracy

Accuracy was estimated as percentage recovery at each addition level and was calculated as the difference between the measured and theoretical value. The % average recovery was determined as 99.673% (Table 3).

Precision

Three concentrations (20, 40, and 60 µg/mL) of drug solution were analysed in triplicates for intra-day and inter-day precision.

There was no significant variation in repeatability in intra-day (% RSD 0.560) and inter-day (% RSD 0.548 - 0.586) estimations. The obtained values were below 2% (Table 2).

Robustness

It indicates that the small changes performed in flow rate, wavelength detection, and column temperature, have no significant effect on the peak area ratio. So, the developed method was found to be robust and %RSD remained below 2.0% (Table 4).

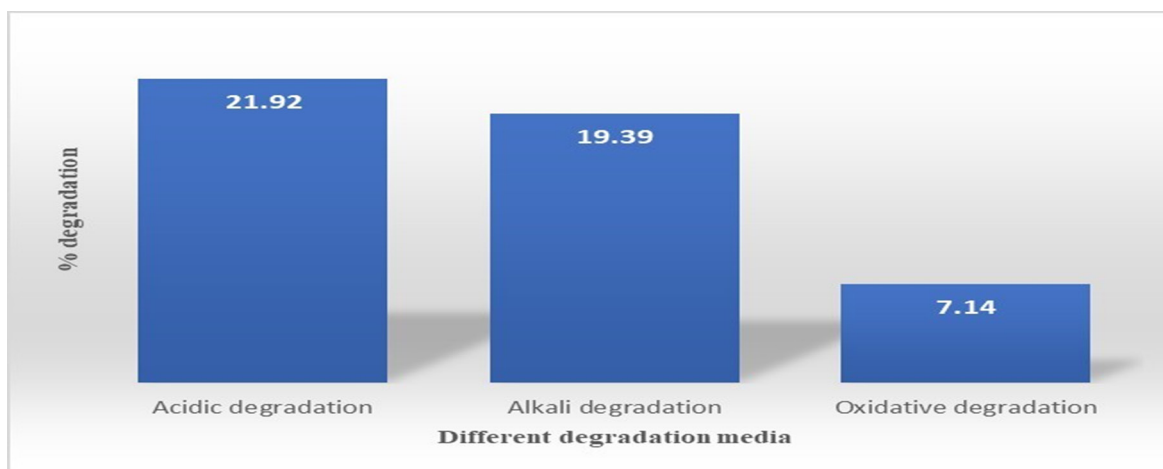


Figure 5: Percentage degradation under different stress conditions.

Analysis of marketed formulation

A chromatogram of the Solvin tablets (IPCA Laboratories Ltd.,) revealed only one spot at R_t : 2.7. Estimated drug amount was found to be 99.6% in tablets, which indicated conformity with the label claim.

Degradation studies

Acid-induced degradation

In acidic degradation, one degradant was resolved at R_t 3.58 along with a drug peak at R_t of 2.66, corresponding to 21.92% degradation. (Figures 4A, 5, and Table 5).

Base-induced degradation

In the study, 19.39% degradation was observed within 1 hr. One degradation product was resolved at R_t 3.8 (Figures 4B, 5, and Table 5).

Oxidative degradation

In oxidative degradation one degradant was obtained at R_t 3.7, resulting in 7.14% degradation (Figures 4C, 5, and Table 5).

Thermal and photodegradation

The study of thermal degradation was performed at 60°C for 4 hr, while in photochemical degradation, PHE was exposed to direct sunlight for 48 hr, but under both conditions, no degradation product was detected.

DISCUSSION

A specific and precise HPLC method of stability indicating was developed for estimating phenylephrine hydrochloride in bulk and tablet by using Luna® 5 μ m C₁₈ column and 5mM ammonium acetate (pH 4.7): methanol (80:20; v/v) as the mobile phase at wavelength of 272 nm. The retention time of PHE was obtained at

2.6 min. The linearity was obtained at five data points giving best correlation. In precision no significant variation was found for inter and intra-day having values of % RSD below 2%. Detection limit and quantitation limit were found to be 6.1658 and 18.6843 μ g/mL respectively, which indicated sensitivity of the method. The % recovery was found to be 99.673, which shows that the developed method is accurate. The aimed method was found to be robust, specific, and accurate.

In degradation studies drug was found to be sensitive to acidic, basic, and oxidative stress conditions resulting one degradation product in each of above stress conditions but was found to be stable under thermal and photochemical degradation conditions. Overall three degradation products were detected.

CONCLUSION

To estimate PHE and its degradation products, a precise and accurate method of RP-HPLC stability indicating was developed. The aptly validated method was able to analyse the drug from its degradant according to the guidelines of ICH. The experimental study revealed that PHE was stable to thermal, and photochemical stress and sensitive to hydrolytic and oxidative stress. The aimed method was fast, accurate, responsive, and specific. Stability data presented in the proposed work may provide great help in product development, and stability studies. It may also provide great commercial value for the industries in the analysis of the drug and its tablet dosage formulation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PHE: Phenylephrine hydrochloride; **UV:** Ultraviolet; **HPLC:** High performance liquid chromatography; **UPLC:** Ultra performance liquid chromatography; **HPTLC:** High performance thin layer chromatography; **ICH:** International conference of harmonization; **µg:** Micro gram; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **SD:** Standard deviation; **RSD:** Relative standard deviation.

SUMMARY

The proposed stability indicating HPLC method was validated as per ICH guidelines and the parameters like correlation coefficient (0.9965), % RSD for intra and inter-day precision < than 2% and 99.67% recovery of the method were found within the acceptable limits of the ICH guidelines. Total three degradation products were resolved after forced degradation of phenylephrine. It was found to be sensitive to hydrolytic, oxidative stress conditions. So, the aimed method can be used for routine analysis as well as for stability testing of PHE.

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