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THE DEVELOPMENT AND VALIDATION OF A NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF BROMHEXINE HYDROCHLORIDE AND PHENYLEPHRINE HYDROCHLORIDE IN THEIR TABLET DOSAGE FORM

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Abstract:

A novel, specific, sensitive, accurate and economical stability indicating RP-HPLC method was developed for the estimation of Phenylephrine hydrochloride and Bromhexine hydrochloride in their tablet dosage form. Separation was achieved on C18 column (250 × 4.6 mm, 5µm) using Optimized Mobile Phase Water: Acetonitrile (60:40 % v/v) (pH adjusted to 4.5 with 1% OPA) and flow rate maintained at 1.0 ml/min was used. Wavelength was monitored at 215 nm. Both the drugs were subjected to acid, base, oxidation, thermal and photolytic degradation conditions. The retention time of PHN and BHX were found to be 3.24 min and 7.11 min respectively. The linearity response was observed in range of 5-25 µg/ml and 4-20 µg/ml of PHN and BHX respectively. Force degradation study revealed that maximum degradation of PHN and BHX was occur in Photolytic degradation. (Standard 19.88%, Sample 18.07% and standard 19.81%, Sample 18.26%) respectively. % RSD was found to be less than 2 in precision, Robustness, LOD and LOQ. % recovery was found in range 98-102%. The proposed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Keywords: Phenylephrine hydrochloride, Bromhexine hydrochloride, RP-HPLC, Force Degradation Study.

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INTRODUCTION:

Phenylephrine hydrochloride is chemically Benzenemethanol-3-hydroxy α ((methyl amino) methyl)-1-hydrochloride (alpha R). It is a sympathomimetic agents or (α1–adrenoceptor agonist). They cause local vasoconstriction, thereby reducing congestion and edema of the nasal mucosa. They are used to advance nasal obstruction in the common cold, sinusitis, fever, acute or chronic rhinitis, allergies of the upper respiratory tract.2-3 The chemical structure of Phenylephrine hydrochloride is shown in figure 1. Chemically Bromhexine hydrochloride is 2, 4- Dibromo [[cyclohexyl (methyl) amino] methyl] aniline hydrochloride. It is a Mucolytic agent or expectorant are drugs believed to increase bronchial secretion or reduce its viscosity, facilitating its removal by coughing. The chemical structure of Bromhexine hydrochloride is shown in figure 2. Both drugs are official in Indian pharmacopeia, British Pharmacopeia and United States Pharmacopeia. The combination of PHN and BHX is used in treatment of Congestion with Excessive Mucus as compared to single drug. Literature review reveals that different Spectrophotometric and Chromatographic methods have been reported for estimation of Phenylephrine hydrochloride alone and in combination with other drugs and for Bromhexine hydrochloride and its combination with other drugs. Published UV method for both drug PHN and BHX combination. So, there is need to develop and validated RP-HPLC method and degradation study for these drugs. So, Aim of present work is Development and Validation of Stability indicating assay method for estimation of Phenylephrine hydrochloride and Bromhexine hydrochloride in their combined dosage form. For this purpose marketed tablets Solvin Decongestant Tablet containing 10 mg of PHN, 8 mg of BHX was used.

MATERIALS AND METHODS:

2.1. Instrumentation

The chromatography was performed on a RP-HPLC instrument equipped with UV detector and RP C_{18} column (250 mm × 4.6 mm, 5µm) was used as stationary phase. Swisser analytical balance, pH meter, an ultrasonic cleaner (Toshcon), Hot air oven.

2.2. Reagent and chemicals

Pharmaceutically pure sample of PHN and BHX were obtained as a gift samples from Manish Pharma Lab, Viramgam and Espee Formulation Pvt. Ltd, Rajkot. All solvents were of HPLC and AR grade obtained from Merck, Rankem and Chemdyes, Rajkot respectively. 2.3. Chromatographic Condition Separation was achieved by RP C18 column (250mm×4.6 mm, 5µm) as stationary phase with water: acetonitrile (60:40 % v/v) as a mobile phase and pH of 4.5 adjusted with 1% orthophosphoric acid at a flow rate of 1 ml/min. Wavelength was monitored of PHN and BHX at 215 nm with UV detector and 20 µL injection volume.

2.4. 1. Preparation of Stock Solution

Accurately weighed 10 mg of PHN and 8 mg of BHX taken into two different 100 ml volumetric flask and make up volume with methanol (100µg/ml of PHN and 80µg/ml BHX).

2.4.2. Preparation of Working Solution

PHN from stock solution pipetting out 1.5 ml and diluted up to 10 ml with methanol (15µg/ml). BHX From stock solution pipetting out 1.5 ml and diluted up to 10 ml with methanol (12µg/ml).

2.4.3. Preparation of Calibration Curve

The calibration curves were plotted over a concentration range of 5-25 µg/ml for PHN and 4-12 µg/ml for BHX. pipetting out 0.5, 1, 1.5, 2, 2.5 ml from stock solution (100 µg/ml of PHN and 0.5, 1, 1.5, 2, 2.5 ml from stock solution 80 µg/ml of BHX) into 10 ml volumetric flask and make up the volume up to the mark with methanol.

2.5. Forced Degradation Study

2.5.1. Sample Preparation for Acid Degradation

Acid decomposition study was performed by refluxing the working solution of both drugs (1.5 ml) in 2 ml of 0.1N HCl for 3 hr at 70 ºC. After 3 hr solutions neutralized with 2 ml of same strength of base (0.1 N NaOH) and finally make up to 10 ml volume with methanol, sonicated and filtered through 0.45µm membrane filter paper and injected in to HPLC system.

2.5.2. Sample Preparation for Base Degradation

Alkali decomposition study was performed by refluxing the working solution of both drugs (1.5 ml) in 2 ml of 0.1 N NaOH for 4 hr at 70 ºC. After 4 hr solution neutralized with 2 ml of same strength of 0.1 N HCl and finally make up to 10 ml volume with methanol, sonicated and filtered through 0.45µm membrane filter paper and injected in to HPLC system.

2.5.3. Sample Preparation for Oxidative Degradation

Oxidative decomposition study was performed by refluxing the working solution of both drugs (1.5 ml) in 2 ml 3% H2O2 for 3 hr at 70 ºC. After 3 hr volume make up to 10 ml with methanol, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system.

2.5.4. Sample Preparation for Thermal Degradation

Thermal decomposition study was performed by refluxing the working solution of both drugs (1.5 ml) for 30 min at 105 ºC. After 30 min volume make up to 10 ml volume with methanol, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system.

2.5.5. Sample Preparation for Photolytic Degradation

Photolytic degradation was performed by exposing the working solution of both drugs (1.5 ml) to Sunlight for 5 hr. After time period, volume make up to 10 ml volume with Methanol, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system.

2.6. Analysis of Marketed Formulation

The method was used for simultaneous estimation of PHN and BHX in tablet dosage forms. For the sample preparation Mobile phase was used as a solvent. Ten tablets were powdered, accurately weighed (equivalent to 10 mg) and transferred in to 100 ml volumetric flask, added about 5 ml of Mobile phase in to it, sonicated for 30 minutes with intermittent shaking, cooled to attain room temperature and added up to 1200ml of Mobile phase and mixed well. It was filtered through 0.45 µ syringe filter. Further 1.5 ml of the above filtrate was diluted to 10 ml with Mobile phase to get 15 µg/ml concentration of PHN and 12 µg/ml concentration of BHX in mixture sample respectively. Absorbance of sample solution was measured at all selected wavelength.

The content of PHN and BHX in sample solution of tablet was calculated.

2.7. Method Validation

2.7.1. Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions of PHN and BHX were compared.

2.7.2. Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5 – 25 µg/ml and 4 – 20 µg/ml for PHN and BHX, respectively. The solutions of each concentration were injected under the operating chromatographic condition as described earlier. Chromatograms were recorded. Plot the calibration curve of Peak area verses concentration and determine Correlation co-efficient and Regression equations for PHN and BHX. These operations were done five times and mean responses were calculated. % RSD was calculated. It should not be more than 2%.

2.7.3. Precision

PHN 15 µg/ml solution and BHX 12 µg/ml solution were analyzed and the absorbance of the each solution was measured six times, absorbance was measured and % RSD was calculated.

2.7.3.1. Intraday precision

Three replicates of three concentrations (5, 15 and 25µg/ml of PHN and 4, 12 and 20µg/ml of BHX), were analyzed at short interval of time, absorbance was measured and % RSD was calculated.

2.7.3.2. Interday precision

Three replicates of three concentrations (5, 15 and 25µg/ml of PHN and 4, 12, 20µg/ml of BHX), were analyzed at three consecutive days and absorbance was measured and % RSD was calculated.

2.7.4. Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by spiking standard drug solution at the level of 80%, 100% and 120% to the pre-analyzed sample solution of PHN and BHX (10 and 8 µg/ml respectively). In this method the known concentration of standard drug was spiked to the assay sample. Each sample was prepared in triplicate at each level and injected. The amount of PHN and BHX were estimated by applying obtained values to the regression equation of the calibration curve. Acceptance criteria: 98 – 102 %.

2.7.5. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by following equations.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of Y- intercept of 5 calibration curves.

S = the mean slope of the 5 calibration curves.

2.7.6. Robustness

Combined standard solutions of PHN (15µg/ml) and BHX (12µg/ml) were prepared and analyzed changing mobile phase, flow rate and pH by measuring the corresponding responses 3 times.

RESULTS AND DISCUSSION:

3.1. Method Development

3.1.1. Optimized Chromatogram Mobile phase Water: Acetonitrile: (60:40%v/v), pH adjusted to 4.5 with 1% Orthophosphoric acid (figure 3).

3.2. Force degradation study

Acid degradation base degradation, oxidative degradation, thermal degradation and photolytic degradation are depicted as chromatograms and given in figure 4, 5, 6, 7 and 8 respectively.

3.3. Analysis of Marketed Formulation

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation named Solvin Decongestant Tablet.

The accuracy of the method was determined by calculating the recoveries of PHN and BHX by the standard addition method at three concentration levels (80, 100 and 120%). The percentage recoveries of PHN and BHX were found to be in the range of 99.38 – 99.43% and 99.21 – 101.24% respectively (Table 2). Percentage Assay of PHN and BHX were found to be in an acceptance limit so this method can be used for analysis of this combination.

3.4. Validation of Stability Indicating RP-HPLC Method

3.4.1. Specificity

In the specificity study, blank, standard and sample were injected into the system. The chromatograms of blank do not show any interference at the retention time of PHN and BHX as it can be seen from respective chromatograms. Chromatographic condition of diluent was shown that there is no interference from the diluent (figure 9, 10).

3.4.2. Linearity

The linearity study was carried out for both drugs at different concentration levels. The linearity of PHN and BHX was in the range of 5 - 25 µg/ml and 4 - 20 µg/ml respectively. The % RSD of all results were less than 2%.The r^2 value was found 0.999 for both the drug (figure 11, 12 and13) and (Table 3 and 4).

3.4.3. Precision

For, Repeatability, % RSD of PHN was found to be 1.06 %, while for BHX, it was found to be 0.81 % (Table 5)

3.4.3.1. Intraday Precision

For, Intraday precision, % RSD of Phenylephrine hydrochloride was found to be 0.60 – 1.06 %, while for Bromhexine hydrochloride, it was found to be 0.69 – 0.93 % (Table 6).

3.4.3.2. Interday Precision

For, Interday precision, % RSD of Phenylephrine hydrochloride was found to be 0.79– 1.80 %, while for Bromhexine hydrochloride, it was found to be 0.94 – 1.53% (Table 7). For Repeatability, Intraday precision and Interday precision, % RSD for both drugs were found to be less than 2. So, it can be concluded that proposed method for estimation of PHN and BHX is precise in nature.

3.4.3.3. Accuracy (Standard Addition Method)

Accuracy of the method was confirmed by recovery study from marketed formulation at three level 80%, 100% and 120 % of standard addition. For, Accuracy, % Recovery for PHN was found to be 99.77–101.54 %, while for BHX, it was found to be in range of 99.80–100.55% (Table 8 and 9). Result obtained reveals that % recovery of PHN and BHX were within acceptance criteria given in ICH guideline i.e. 98-102%.

3.4.3.4. Limit of Detection and Limit of Quantification

Calibration curve was repeated for 5 times and the standard deviation (S.D) of the intercepts was calculated. Then LOD and LOQ were measured as follows (Table 10).The proposed method can detect and quantify small amount of drugs with precision. So, it can be concluded that the proposed method is very sensitive in nature.

3.4.3.5. Robustness

Varying conditions of temperature, pH and mobile phase composition were carried out and % RSD was found less than 2% (Table 12).

CONCLUSION:

A novel, specific, sensitive, accurate and economical stability indicating reversed-phase highperformance liquid chromatographic method was developed for the estimation of Phenylephrine hydrochloride and Bromhexine hydrochloride in their tablet dosage form.

Both the drugs were subjected to acid and base hydrolysis, oxidation, thermal and photolytic degradation conditions.

Maximum degradation of Phenylephrine hydrochloride was occur in Photolytic degradation. Standard (19.88%) and Sample (18.07%).

Maximum degradation of Bromhexine hydrochloride was occur in photolytic degradation. Standard (19.81%) and Sample (18.26%).

Validation parameters prove that method is repeatable, sensitive and selective for the analysis of Phenylephrine hydrochloride and Bromhexine hydrochloride in tablet dosage form.

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