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A NEW CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF DOXYLAMINE SUCCINATE, PYRIDOXINE HYDROCHLORIDE AND RELATED IMPURITIES

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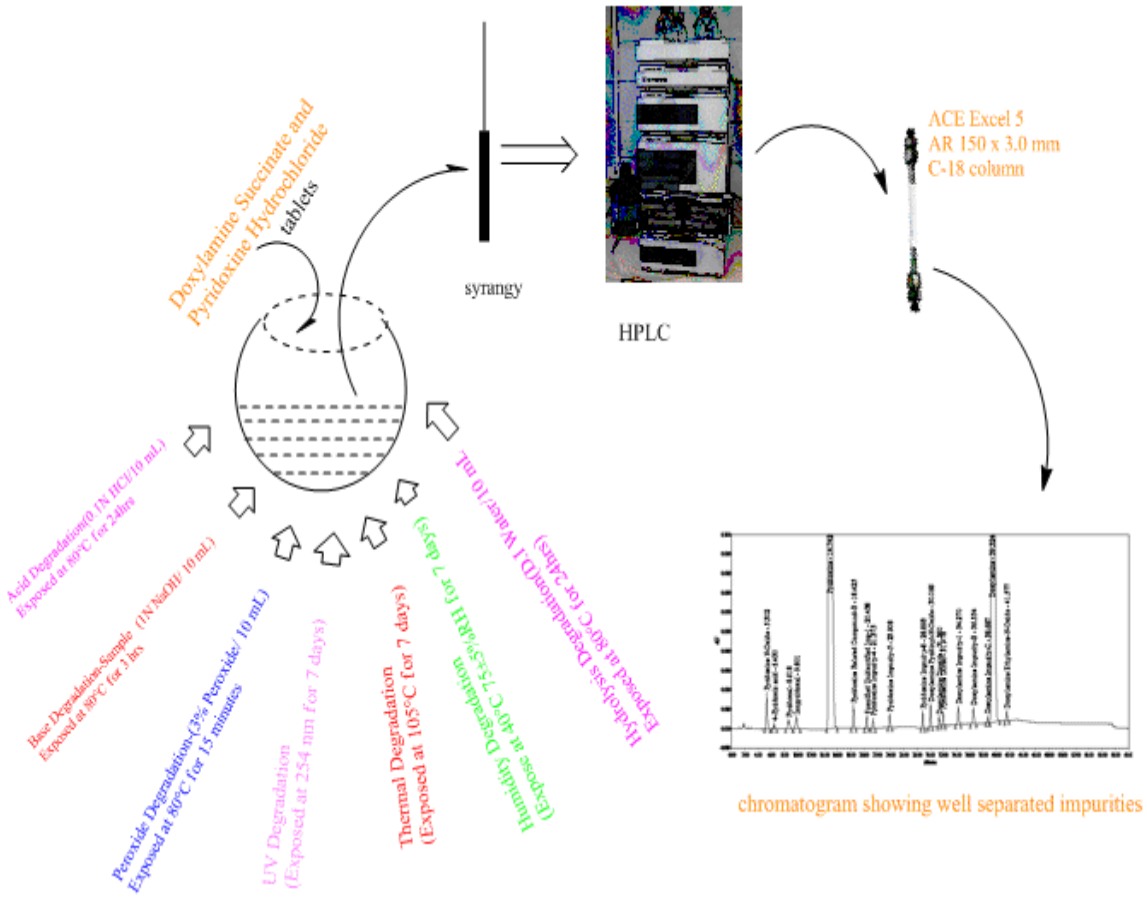
ABSTRACT

The combination of Doxylamine Succinate and pyridoxine Hydrochloride wasrecognized for the treatment of nausea and vomiting in pregnancy women. The present investigation illustrates a new rapid, precise, accurate, stability indicating reverse phase high performance liquidchromatographic separation methodology was developed for highly resolved separation and determination of Doxylamine Succinate, Pyridoxine Hydrochloride and related nineteen impurities in Pharmaceutical dosage forms among them 14 impurities are degraded quantified 4 process related impurities were separated and unknown impurity was separated but not quantified. Chromatographic separation is achieved on an ACE Excel 5 AR 150 x 3.0 mm C-18 column using gradient elution. Mixture of Di ammonium hydrogen phosphate-n-pentane sulfonic acid buffer (P^H=4.00±0.05), Methanol and n-propanol in a ratio of 400: 525: 75v/v/v respectively was used as mobile phase. The eluted compounds were monitored at 268 nm; the flow rate was 0.7 ml/min and column oven temperature maintained at 25±2°C. Resolution of determination of doxylamine succinate and pyridoxine hydrochloride and impurities(potential, bi-products and degradation) is greater than 2.0 for all pair of components. The high correlation coefficient (r^2> 0.999) values indicated good correlations. The repeatability and intermediate precision, expressed by the RSD was less than 1.5%. The performance of the method was validated according to the present ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness. Stress studies and Peak purity studies were carried out according to ICH guidelines. All the degradation product (s) arose by induced degradation were well separated from the drug peaks. The degradation peaks observed due to various extreme conditions (such as acidic, alkaline, oxidation, hydrolysis, photolytic, thermal, etc.) are completely resolved from main drug peak. Hence the method found to be stability indicating. The proposed method is practical and valuable for its routine application in quality control laboratories for estimation of Doxylamine Succinate, Pyridoxine Hydrochloride and related nineteen impurities in Pharmaceutical dosage forms.

GRAPHICAL ABSTRACT



New chromatographic method for simultaneous separation of Doxylamine Succinate, Pyridoxine Hydrochloride and related impurities in Delayed-Release Tablets 10mg/10mg using ACE Excel 5 AR 150 x 3.0 mm C-18 column.

1. INTRODUCTION

Doxylamine succinate, (RS)-N,N-dimethyl-2-(1-phenyl-1-pyridin-2-yl-ethoxy)-thanamine, is chemically used as an antihistaminic drug and anti-muscarinic agent and sedative effect.[1,2] It exhibits moderate alkaline nature with a pKa of 8.8; apart from this, it is commercialized as succinate salt is one of the first-generation H1 receptor antagonists of the ethanolamine group.[3–5] It belongs to counter sedatives to treat insomnia having decongestants and antitussives to mitigate symptoms of cough and cold in a patient. 3,4-Pyridinedimethanol,5-hydroxy-6-methyl-hydrochloride. The Chemical formula is $C_8H_{11}NO_3 \cdot HCl$. The Molecular weight is 205.64 and chemical structure is as shown below. The combination of DOX and pyridoxine approved by the American College of Obstetricians and Gynecologists for administration was recognized for the treatment of nausea and vomiting.[6–8]

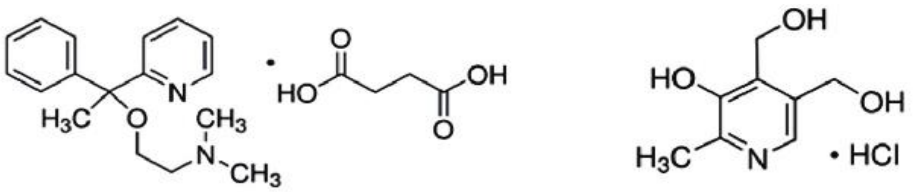


Figure 1: Chemical Structure of Doxylamine Succinate and Pyridoxine Hydrochloride.

Usually, for product advancement, stability examination plays a crucial part moreover product performance and its quality mainly depend upon various factors such as temperature, pH, humidity, and light. It also facilitates authorized self life's suggested storage condition. For drug product development, active drug and product degradant play a significant role.[9–11] However, there is a growing concern against the progress of stress testing using stability-indicating assay method as this assimilated in International Conference on

Harmonization (ICH) guidelines. This path is being used in the amalgamation of drugs useful in the existence of degradant products.[12] As previously described, methods such as UV spectrophotometric[13–16], HPLC[17–21], HPTLC[22], GC[23], FT-IR[24,25], and capillary electrophoresis[26] were only reported.

Developing and optimizing isocratic HPLC techniques[27] could be a sophisticated practice that needs synchronized fortitude of several factors. HPLC methods were optimized by time-consuming trial-and-error approach for the last several years, resulting only in an obvious optimum and information regarding the sensitivity of the factors on analytes separation and interaction between factors is not available. Hence, any one of the chemo metric methods which includes the overlapping resolution maps, factorial design and response surface methodology[28–31] can be useful.

Keeping all the above facts in a view in the present research investigation, first time in the literature, the author works upon reverse phase high performance liquid chromatographic method (RP-HPLC) for determination degradation products and to establish the fact that the inherent chemical stability of the Doxylamine succinateand PyridoxineHCl tablets, remains intact during its existence. If any degradation has occurred, it can be monitored and resolved to quantify the nature and extent of degradation.

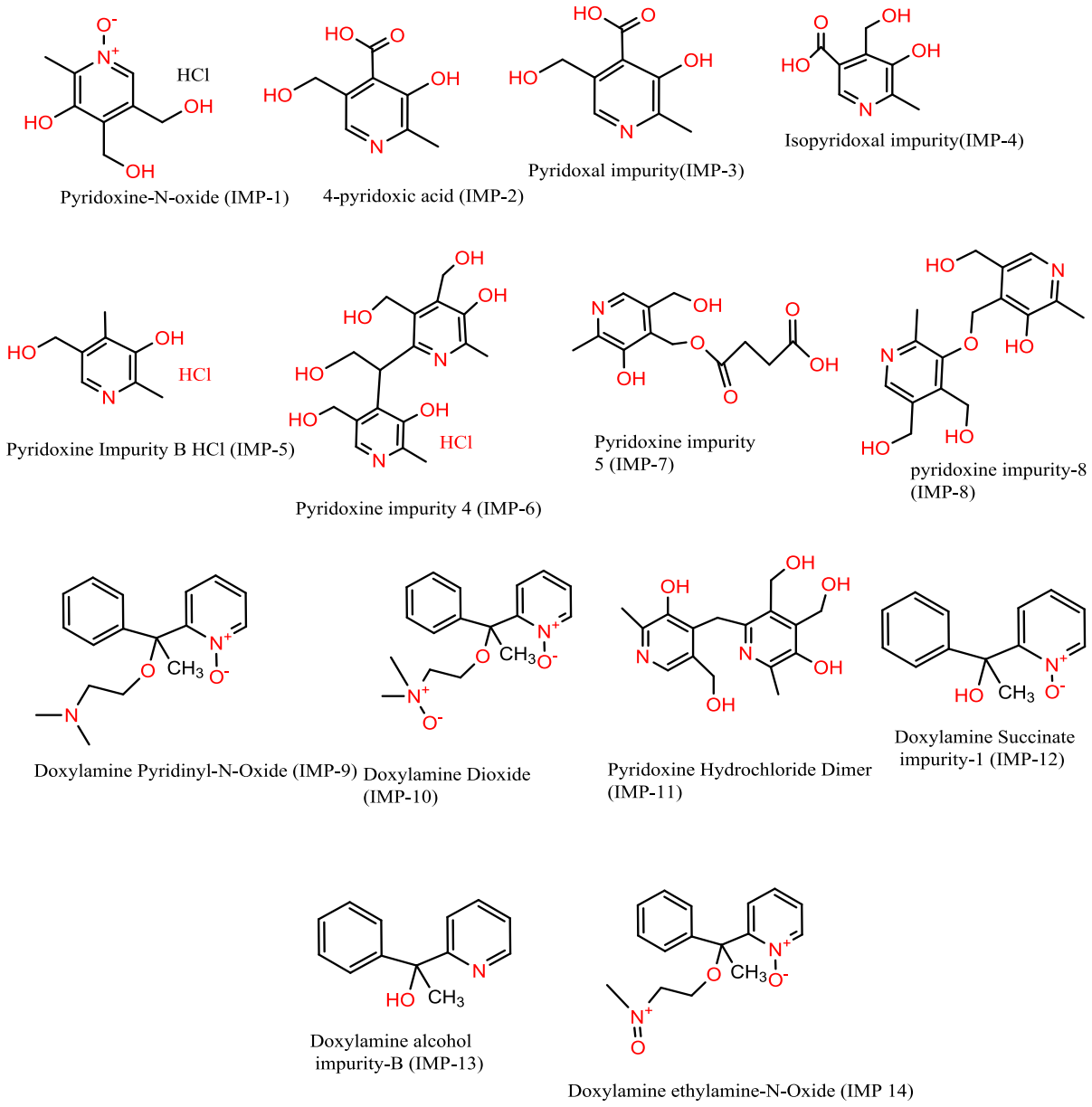


Fig:2. Structures of Doxylamine Succinate and pyridoxine Hydrochloride impurities those were well separated and quantified

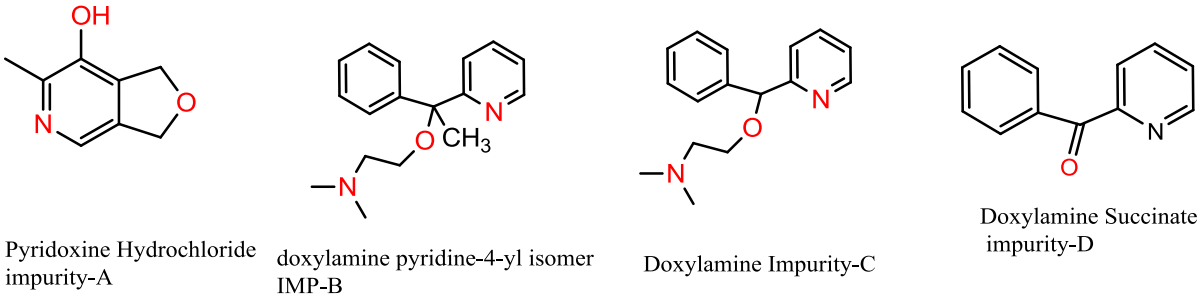


Figure:3, Process related impurities of Doxylamine Succinate and pyridoxine Hydrochloride

2.0. METHOD DEVELOPMENT

There are limited papers are available on separation of Pyridoxine hydrochloride, Doxylamine succinate and its related compounds for combination drug product. Initial development trails were taken on different stationary phases on different mobile phase compositions without any ion pair in the mobile phase and separation at pyridoxine and its related compounds were not achieved on any of the stationary phase as pyridoxine retention factor is less than 2. To increase the retention factor for pyridoxine, introduced alkylsulfonate as ion pair in the mobile phase. The purpose of adding an ion pair reagent to the mobile phase is to change the retention time of ionic analyte. By varying the length and concentration of ion pair reagent, the retention factor charge analyte can be continuously increased by factor of 5-25 compare to the value with no addition pair reagent. After many development trails, the separation of pyridoxine, doxylamine and its related compounds were achieved on C18 stationary phase with n-pentane sodium sulfonate as ion pair reagent.

2.1. Apparatus

HPLC System with UV/PDA Detector (Waters), Analytical Balance(Sartorius), Analytical micro balance(Mettler Toledo), Sonicator (Bransonic), ACE Excel 5 AR 150 x 3.0 mm C-18 column(Mack mode), Centrifuge(Labnet)

2.2. Reagents and standards: De-ionized water (In-House)**,** 1-propanol (HPLC grade)**,** Doxylamine Succinate reference/working standard**,** Pyridoxine Hydrochloride reference/working standard**,** Di ammonium hydrogen phosphate (ACS Reagent Grade)**,** 1-Pentane sulfonic Acid Sodium Salt monohydrate (Macron/HPLC Grade)**,** Phosphoric acid (J.T baker/ Reagent grade)**,** Methanol (J.T baker/HPLC grade)**,** Hydrochloric acid (ACS Reagent grade)**,** Pyridoxine Hydrochloride reference/working standard**,** Doxylamine succinate reference/working standard, Pyridoxine N-Oxide Hydrochloride Impurity,4-Pyridoxic acidImpurity, Pyridoxal Hydrochloride impurity, Pyridoxine hydrochloride impurity- A, Pyridoxine hydrochloride Impurity-B, Pyridoxine hydrochloride Impurity-4,Pyridoxine hydrochloride Impurity-5,Pyridoxine hydrochloride Impurity -8,Doxylamine Pyridine N-Oxide Impurity, Doxylamine Pyridine N-Oxide Impurity, Doxylamine Dioxide Impurity, Pyridoxine hydrochloride Dimer Impurity, Doxylamine Impurity-1, Doxylamine Succinate Impurity-A, Doxylamine Succinate Impurity-B, DoxylamineImpurity -C(Carbinoxamine related compound-C), Doxylamine ethylamine -N-Oxide and Doxylamine Impurity -D

2.3. Mobile Phase-A: Weigh about 1.0 g of di ammonium hydrogen phosphate and about 1.2 g of n-pentane sulfonic acid sodium salt monohydrate (Macron/HPLC Grade), dissolve in 1000 mL of D.I water by sonication and mix well. Adjust the pH of the solution to 4.00±0.05 with phosphoric acid and mix well. Filter the solution through 0.45micron membrane filters and degas.

2.4. Mobile Phase-B: Buffer Preparation for Mobile Phase-B: Weighed about 1.0 g of di ammonium hydrogen phosphate (Sigma Aldrich/HPLC Grade), dissolve in 1000 mL of D.I water by sonication and mix well. Adjust the pH of the solution to 3.50±0.05 with phosphoric acid (J.T baker/HPLC Grade) and mix well. Filter the solution through 0.45-micron membrane filters and degas.
Mix Buffer, Methanol and n-propanol in a ratio of 400: 525: 75v/v/v respectively

2.5. Diluent: Dilute 17 mL of Hydrochloric acid to 2000 mL with D.I water and mix well.

2.6. HPLC conditions: Column:ACE Excel 5 AR 150 x 3.0 mm: Flow:0.7 mL/min: Injection volume:50µL; Detection wave length:210 nm; Column oven temperature:25^{o}C±2°C; Sample Temperature: 5°C±2°C; Run time :60 Minutes; Elution: Gradient; Sampling Rate:1 point/ second, Needle Wash:20:80v/v (water: Acetonitrile v/v), Seal Wash: Mix D.I water: Acetonitrile in the ratio of 800:200 v/v**.**

3.0. PREPARATION OF SOLUTIONS

3.1. Stock standard preparation

Weigh accurately about 10 mg of Doxylamine Succinate reference standard/working standard and 10 mg of Pyridoxine hydrochloride reference standard/working standard and quantitatively transfer into a 100.0 mL

volumetric flask with the aid of Diluent. Dilute to approximately about 75% volume of the flask with Diluent. Sonicate the flask with occasional shaking to dissolve the solids. Allow the solution to equilibrate to room temperature. Dilute to volume with diluent and mix well. The concentration of stock standard solution is 0.1 mg/mL (100µg/mL) of Doxylamine Succinate and 0.1 mg/mL (100µg/mL) of Pyridoxine Hydrochloride.

3.1.1. Low level standard preparation (0.5% level)
Transfer 1.5 mL of standard stock solution in to a 100 mL volumetric flask and diluted to volume with diluent and mix well. The concentration of diluted standard solution is 0.0015mg/mL (1.5µg/mL) of Doxylamine Succinate and 0.0015 mg/mL (1.5µg/mL) of Pyridoxine Hydrochloride.

3.1.2. Low level standard preparation (0.5% level)
Transfer 1.5 mL of standard stock solution in to a 100 mL volumetric flask and diluted to volume with diluent and mix well. The concentration of diluted standard solution is 0.0015mg/mL (1.5µg/mL) of Doxylamine Succinate and 0.0015 mg/mL (1.5µg/mL) of Pyridoxine Hydrochloride.

3.1.3. Pyridoxine hydrochloride related compound-a stock solution
Weigh accurately about 1 mg of Pyridoxine Hydrochloride related compound-A reference standard/working standard and quantitatively transfer into a 25.0 mL volumetric flask with the aid of Diluent. Dilute to approximately about 75% volume of the flask with Diluent. Sonicate the flask with occasional shaking to dissolve the solids. Allow the solution to equilibrate to room temperature. Dilute to volume with diluent and mix well. The concentration of pyridoxine hydrochloride related compound-A stock standard solution is 0.04 mg/mL (40µg/mL).

3.1.4. Doxylamine succiante related compound-c or carbinoxamine related compound-c stock standard solution
Weighed accurately about 1 mg of Doxylamine succinate related compound-C or Carbinoxamine related compound-C reference standard/working standard and quantitatively transferred into a 25.0 mL volumetric flask with the aid of Diluent. Dilute to approximately about 75% volume of the flask with Diluent. Sonicated the flask with occasional shaking to dissolve the solids. Allowed the solution to equilibrate to room temperature. Diluted to volume with diluent and mix well. The concentration of Doxylamine succinate related compound-C stock standard solution is 0.04 mg/mL (40µg/mL).

3.1.5. Resolution solution preparation
Transferred 5 mL of standard stock solution, 2.0 mL of Pyridoxine Hydrochloride related compound-A stock solution and 3.0 mL of Doxylamine succinate related compound-C stock solution in a 10 mL into volumetric flask and mix well. The concentration of resolution solution is 0.05 mg/mL (50µg/mL) of Doxylamine Succinate, 0.05 mg/mL (50µg/mL) of Pyridoxine Hydrochloride, 0.008 mg/mL (8µg/mL) of Pyridoxine Hydrochloride related compound-A and 0.012 mg/mL (12µg/mL) of Doxylamine succinate related compound-C.

3.1.6. Sample preparation for related compounds
Weighed accurately 20 tablets and determine Average Tablet Weight (ATW) in mg. Crush the tablets into fine powder. Weighed sample equivalent to 30 mg of Doxylamine succinate and transfer it into individual 100.0 mL volumetric flasks with the aid of diluent. Diluted to approximately about 75% volume of the flask with diluent. Sonicated the flasks with occasional shaking for 20 minutes. Allow the solution to equilibrate to room temperature. Dilute to volume with diluent and mix well. Filtered a portion of the solution through 0.45µm PFTE L filter prior to injection the sample by discarding first few mL of filtrate.

4.0. PROCEDURE

Inject diluent as blank twice. Inject detectability level standard solution. Perform Signal / Noise (S/N) calculation for Pyridoxine and Doxylamine peak in detectability level standard solution. If S/N is less than 10, a system with better detectability is required. If the S/N is ≥ 10, make one injection of resolution solution followed by six consecutive injections of low-level standard preparation. Perform the following calculations to determine the system suitability. The resolution between Pyridoxine peak and pyridoxine related compound-A peak is NLT 2.0 and the resolution between Doxylamine related compound-C and Doxylamine peak is NLT 2.0 in resolution solution. The percentage relative standard deviation (%RSD) for Doxylamine peak and Pyridoxine peak areas from six consecutive injections of Low level standard solution should be not more than 5.0 The tailing factor for Doxylamine and Pyridoxine peaks in all injections of Low level standard solution should be not more than 2.0**.**Once the system suitability is attained, inject the sample preparation(s) once. As an ongoing check, perform the following.

Inject two injections of low-level standard solution after every 12 sample injections include two blank injections before check standards every time. Inject two injections of blank injections followed by two injections of low-level standard solution at the end of sample injections.

5.0. ANALYTICAL METHOD VALIDATION

5.1. Specificity

Specificity of analytical method demonstrates its uniqueness for the analyte of interest while quantification in selective manner. All the individual impurities and others possible interferences were injected at 0.5µg mL^{-1}. All the analyte peaks eluted at different retention times without interfering with Doxylamine Succinate and Pyridoxine Hydrochloride Tablets and other impurities

peaks. Further, peak purity of the impurities spiked solution and real time samples were assessed using HPLC-PDA detector. The peak purity results (Purity angle< Purity threshold) showed that Doxylamine Succinate and Pyridoxine Hydrochloride peak is homogeneous and no peak eluting at the retention time of Doxylamine Succinate and Pyridoxine Hydrochloride. Results were given in the table -1 and table -2

5.2. Accuracy

Accuracy test solutions of Doxylamine Succinate and Pyridoxine Hydrochloride (300 µg mL^{-1}) spiked with impurities at LOQ (0.05 µg mL^{-1}), 0.2 µg mL^{-1}0.5 µg mL^{-1} and 0.75 µg mL^{-1} were injected along with control sample and calculated amount found in µg mL^{-1}. Recoveries were calculated (Table 4) against amount added in µg mL^{-1}, in percentage (%) form. Accuracy of the Doxylamine Succinate and Pyridoxine Hydrochloride determination. The mean recovery values (n = 6) for impurities and drug were within the range 84.0% to 105.8% with RSD range from 0.07% to 3.29% indicating that the method is accurate.Results were given in the table -1 and table -2

5.3. Precision

Test solutions of Doxylamine Succinate and Pyridoxine Hydrochloride(300 µg mL-1) spiked with impurities at LOQ (0.05 µg mL^{-1}), 0.2 µg mL^{-1}0.5 µg $mL^{-1}$and 0.75 µg mL^{-1} were injected and calculated %RSD values for recoveries. Precision of Doxylamine Succinate and Pyridoxine Hydrochloride. The RSD were calculated (Table 5). The mean RSD values (n = 6) for impurities and Doxylamine Succinate and Pyridoxine Hydrochloride were within the range 0.05% to 3.73%. Results were given in the table -1 and table -2

5.4. Linearity

Linearity solutions were prepared at eight impurity concentrations (LOQ, 0.10, 0.20, 0.30, 0.50 and 0.75µg mL^{-1}). The calibration curve was drawn by plotting the average analyte peak area for triplicate injections against the concentration. Similarly, the linearity of Doxylamine Succinate and Pyridoxine Hydrochloride. The results for the correlation coefficient (r) and coefficient of determination (R^2) were shown in the Table 5. Relative response factors of impurities were established from linearity method by calculating slopes from the linear regression equation and comparing against Doxylamine Succinate and Pyridoxine Hydrochloride slope. The results were between 0.8 to 1.2 for all the impurities. Results given in the table -1 and table -2

5.6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method the experimental conditions were deliberately changed. The resolution of Doxylamine Succinate and Pyridoxine Hydrochloride and all impurities were evaluated. The mobile phase flow rate was 0.70 ml/min; to study the effect of flow rate on resolution it was changed to 0.65 and 0.75 ml/min. The effect of column temperature was studied at 20°C and 28 °C (instead of 25°C), mobile phase pH was changed ±0.1 and mobile phase composition was changed ±5% variation from the initial composition all Impurities were well separated.

5.7. Solution Stability and Mobile Phase Stability

Doxylamine Succinate and Pyridoxine Hydrochloride spiked solutions (0.5% level) prepared in diluent, by leaving test solutions at room temperature, spiked solutions were injected at 0 h, 24 h, 48h and 72 h of time intervals calculated the impurity content (Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, Imp-9, Imp-10, Imp-11, Imp-12, Imp-13 and Imp-14) and checked the consistency in the % area of the principal peak at each interval. Mobile phase prepared was kept constant during the study period. The mobile phase study was demonstrated by injecting the freshly prepared sample solution at different time intervals. (0 h, 24 h,48h ,72 h and 120h).

5.8. Limit of Detection (LOD) and Quantification (LOQ)

By injecting a linear (0.05% to 0.5% with respect to test concentration) solutions of known concentrations, based on the Standard Deviation (σ) of the Response and the Slope (S) of the calibration plot, using the formula LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$. LOD and LOQ for Doxylamine Succinate and Pyridoxine Hydrochloride and for the impurities were estimated respectively. Precision (n=6) was also determined at LOQ level and % RSD was calculated for the peak area for each impurity and for Doxylamine Succinate and Pyridoxine Hydrochloride. Results were given in the table -1 and table -2

6.0. Induced Degradation Studies

Treated Doxylamine succinate and pyridoxine hydrochloride delayed-release tablets and Doxylamine succinate and pyridoxine hydrochloride delayed-release tablets placebo with the, 1. Acid Hydrolysis (In 10 mL 0.1N HCl at 80°C for 24 hrs), 2). Alkaline hydrolysis (In 10 mL 1N NaOH at 80°C for 3hrs), 3). Oxidation (In 10 mL 3% H_2O_2 at 80°C for 15 min),4). Hydrolysis (In 10 mL D.I water at 80°C for 24 hrs.),4).Open Exposure to UV Light at 254 nm for 7 days. 4). Pyrolysis (Thermal degradation at 105°C for 7 days), 5).Open exposure to humidity condition (40°C/75%RH) for 7Days.

7.0. RESULTS AND DISCUSSION

Table 1: Method validation data of Doxylamine Succinate and Pyridoxine Hydrochloride Tablets and its impurities.

Test	Details	Acceptance Criteria	Results							
			IMP-1	IMP-2	IMP-3	IMP-4	IMP-5	IMP-6	IMP-7	IMP-8
Specificity	*n=1*,each individual impurity at 0.5 µg mL^{-1}	Each peak should elute at different retention time	There are no peaks interferences with Doxylamine Succinate and Pyridoxine Hydrochloride and its impurities. All the individual impurities peaks eluted at different retention times.							
Linearity	6 concentration levels in the range 0.05–0.75 µg mL^{-1}	Correlation coefficient should be > 0.999	0.999	0.998	0.999	0.999	0.999	0.999	0.999	0.999
Limit of detection	0.001 µg mL^{-1} (lowest detectable concentration)	S/N ratio > 3	0.001	0.009	0.005	0.004	0.005	0.005	0.004	0.003
Limit of quantification	0.05 µg mL^{-1} (lowest quantifiable concentration)	S/N ratio > 10	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Accuracy	*n=18* (6 determinations each at LOQ, 0.5 µg mL^{-1}& 0.75 µg mL^{-1}	Recovery at each level should be > 80-120%	102.8 % 102.1 % 102.7% 101.9%	95.2% 99.0% 101.4% 100.5%	85.5% 101.8% 102.2% 101.3%	94.0% 102.8% 102.4% 101.9%	102.0% 102.9% 103.2% 102.3%	105.0% 105.8% 88.9% 94.1%	89.1% 100.1% 101.8% 100.8%	89.7% 97.2% 99.9% 99.7%
Precision (repeatability)	*n=18* (6 determinations each at LOQ, 0.5 µg mL^{-1}& 0.75 µg mL^{-1}	RSD should be < 10% for LOQ RSD should be < 5% for remaining levels	0.58% 0.09% 0.41% 0.22%	3.31% 0.51% 1.43% 0.51%	3.27% 0.59% 0.17% 0.26%	1.66% 0.32% 0.45% 0.19%	0.57% 0.07% 0.24% 0.25%	3.08% 0.99% 0.51% 0.40%	2.18% 0.50% 0.43% 0.24%	0.79% 0.50% 0.19% 0.31%
Intermediate precision	*n=6* (6 determinations at 0.5 µg mL^{-1}	RSD should be <10%	0.54%	1.12%	0.37%	0.49%	0.50%	1.51%	0.99%	0.27%

Table 2: Method validation data of Doxylamine Succinate and Pyridoxine Hydrochloride Tablets and its impurities.

Test	Details	Acceptance Criteria	Results							
			IMP-9	IMP-10	IMP-11	IMP-12	IMP-13	IMP-14		
Specificity	*n=1,* each individual impurity at 0.5 µg mL^{-1}	Each peak should elute at different retention time	There are no peaks interferences with Doxylamine Succinate and Pyridoxine Hydrochloride and its impurities. All the individual impurities peaks eluted at different retention times.							
Linearity	6 concentration levels in the range 0.05–0.75 µg mL^{-1}	Correlation coefficient should be > 0.999	0.999	0.998	0.999	0.999	0.999	0.999		
Limit of detection	0.001 µg mL^{-1} (lowest detectable concentration)	S/N ratio > 3	0.002	0.004	0.002	0.002	0.005	0.005		
Limit of quantification	0.05 µg mL^{-1} (lowest quantifiable concentration)	S/N ratio > 10	0.05	0.05	0.05	0.05	0.05	0.05		

Accuracy	*n=18* (6 determinations each at LOQ, 0.5 µg mL^{-1}& 0.75 µg mL^{-1}	Recovery at each level should be > 80-120%	98.0% 99.7% 101.9% 101.7%	111.1% 102.3% 102.4% 101.8%	106.5% 102.2% 102.0% 100.8%	95.4% 99.3% 101.3% 101.1%	91.9% 99.7% 103.7% 104.8%	84.0% 97.8% 101.7% 100.9%		
Precision (repeatability)	*n=18* (6 determinations each at LOQ, 0.5 µg mL^{-1}& 0.75 µg mL^{-1}	RSD should be < 10% for LOQ RSD should be < 5% for remaining levels	0.46% 0.45% 0.28% 0.31%	2.24% 0.52% 0.28% 1.46%	0.77% 0.28% 0.30% 0.24%	1.03% 0.25% 0.24% 0.23%	1.68% 0.47% 0.48% 0.31%	3.29% 0.98% 0.60% 0.61%		
Intermediate precision	*n=6* (6 determinations at 0.5 µg mL^{-1}	RSD should be <10%	0.75%	1.48%	0.65%	0.74%	0.85%	0.65%		

8.0. Induced degradation

The induced degradation study proved that the method is capable to quantitative the impurities accurately from the peaks obtained from the degradation of drug in acidic, base, peroxide, thermal, UV conditions and Humidity conditions.

Table 3: Induced Degradation details for Doxylamine succinate and pyridoxine hydrochloride delayed-release tablets.

S.No	Test	Stressing Agent/added (mL)	Stressing Conditions	Neutralized with/added (mL)
1	Control-Sample	Not Applicable	Not Applicable	Not Applicable
2	Acid Degradation-Sample	0.1N HCl/10 mL	Exposed at 80°C for 24hrs	0.1N NaOH/10 mL
3	Base Degradation-Sample	1N NaOH/ 10 mL	Exposed at 80°C for 3 hrs	1N HCl/10 mL
4	Peroxide Degradation-Sample	3% Peroxide/ 10 mL	Exposed at 80°C for 15 minutes	Not Applicable
5	UV Degradation-Sample	Not Applicable	Exposed at 254 nm for 7 days	Not Applicable
6	Thermal Degradation-Sample	Not Applicable	Exposed at 105°C for 7 days	Not Applicable
7	Humidity Degradation-Sample	Not Applicable	Expose at 40°C 75±5%RH for 7 days	Not Applicable
8	Hydrolysis Degradation-Sample	D.I Water/10 mL	Exposed at 80°C for 24hrs	Not Applicable

Table 4: Chromatographic data for Doxylamine succinate and pyridoxine hydrochloride delayed-release tablets induced degradation.

S.No.	Sample/Solution name	% of related compounds							
		Control	Acid	Base	Peroxide	Thermal	UV	Humidity	Hydrolysis
1	Pyridoxine N-Oxide	ND	ND	0.02	0.08	0.00	ND	ND	ND
2	4-Pyridoxic acid	ND	ND	ND	ND	0.06	ND	ND	ND
3	Pyridoxal impurity	0.07	0.09	0.08	0.04	0.28	0.06	0.05	0.09
4	IsopyridoxalImpurity	0.02	0.02	0.02	0.06	0.09	0.02	0.02	0.02
5	Pyridoxine Impurity-B	ND	ND	ND	ND	0.02	ND	ND	0.0
6	Specified Unidentified Imp-1	0.01	0.16	0.03	0.03	2.96	0.04	0.11	0.83
7	Pyridoxine Impurity-4	0.02	0.01	0.00	ND	0.74	0.02	0.02	0.00
8	Pyridoxine Impurity-5	0.05	0.02	0.00	0.03	1.72	0.05	0.10	0.00
9	Pyridoxine Impurity-8	ND	ND	0.00	ND	0.04	ND	ND	0.00
10	Pyridoxine Dimer	0.01	0.10	0.54	0.03	0.31	0.02	0.03	2.82
11	Doxylamine Pyridinyl-N-Oxide	ND	ND	ND	0.01	0.02	ND	ND	ND
12	Doxylamine Dioxide	ND	ND	ND	ND	ND	ND	ND	ND
13	Doxylamine Impurity-1	0.00	0.01	0.01	0.02	0.00	0.01	0.01	0.01
14	Doxylamine alcohol (Impurity-B)	0.01	1.15	0.01	0.10	0.33	0.01	0.01	0.03
15	Doxylamine ethylamine-N-Oxide	0.04	0.01	0.78	15.18	0.69	0.04	0.05	0.19
16	Maximum unspecified impurity	0.03	0.03	0.11	0.55	0.55	0.02	0.03	0.07
17	Total % of impurities	0.36	1.75	2.14	16.67	10.27	0.36	0.52	4.27

Table-5: Chromatographic data for Doxylamine succinate and pyridoxine hydrochloride delayed-release tablets + 0.5%level spiked sample.

S.No.	Sample/Solution name	0.5% level spiked sample	
		Retention time (min)	RRT
1	Pyridoxine N-Oxide	5.302	0.36
2	4-Pyridoxic acid	6.430	0.44
3	Pyridoxal impurity	8.618	0.58
4	Isopyridoxal impurity	9.801	0.66
5	Pyridoxine	14.742	1.00
6	Pyridoxine Impurity-B	18.425	1.25
7	Specified Unidentified Impurity-1	20.438	1.39
8	Pyridoxine Impurity-4	21.375	1.45
9	Pyridoxine Impurity-5	23.908	1.62
10	Pyridoxine Impurity-8	28.893	1.96
11	Doxylamine Pyridinyl-N-Oxide	30.066	0.76
12	Pyridoxine Dimer	31.978	0.81
13	Doxylamine Dioxide	31.360	0.80
14	Doxylamine Impurity-1	34.270	0.87
15	Doxylamine alcohol (Impurity-B)	36.554	0.93
16	Doxylamine Impurity-C	38.687	0.98
17	Doxylamine	39.324	1.00
18	Doxylamine ethylamine-N-Oxide	41.577	1.06

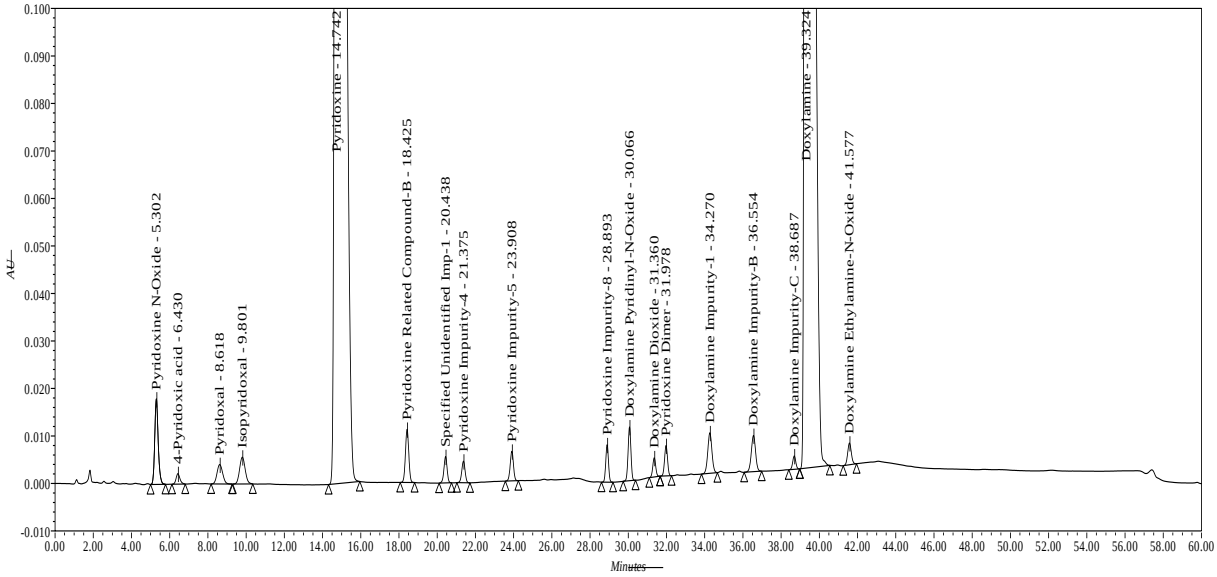


Figure 4: Chromatogram showing well separated impurities of Doxylamine succinate and pyridoxine hydrochloride delayed-release tablets.

9.0. CONCLUSION

Analytical method for analysis of related compounds of Doxylamine succinate and pyridoxine hydrochloride is specific, linear, accurate, precise, robust, rugged and stability indicating and can be used for the analysis of Doxylamine succinate and pyridoxine hydrochloride delayed-release tablets 10mg/10mg. The analytical method is stability indicating and separates all the impurities as well as degradation products arose by induced degradation.

The validation results of the Impurity procedure to determine the % of unknown impurity levels by the HPLC procedure indicate that the method is scientifically sound.

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