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# RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DOXYLAMINE SUCCINATE AND PYRIDOXINE HCL IN ITS PURE AND PHARMACEUTICAL TABLET DOSAGE FORM

**S.Ravichandran [1], S.Selvakumar[2*], Afreen[2], Nuzhat Banu[2]**

[1]P.S.V. College of Pharmaceutical Science and Research, Orappam Village, Bargur (Tk), Krishnagiri (Dt), Tamilnadu –635108, India.

[2]Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally (V), Yadagirigutta (M), Yadadri-bhongir (Dt), Telangana –508 286, India.

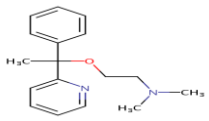
**ABSTRACT**

Doxylamine succinate and Pyridoxine HCL was carried out by RP-HPLC method on a Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters) using a mobile phase consisting of pH 3.5 phosphate buffer: Acetonitrile (30:70). The mobile phase was pumped at a rate of 1.0 ml/min and the detection was carried out at 254nm. The retention time of Doxylamine succinate and Pyridoxine HCL was found to be 2.162 and 3.305 min respectively and linearity was in the range of 12-60µg /ml for Pyridoxine HCL and 20-100µg/ml for Doxylamine succinate. The results obtained in newer RP-HPLC method for determination of Doxylamine succinate and Pyridoxine HCL are tabulated and also discussed about the developed RP-HPLC method. The proposed method is simple cost effective and gives reliable assay results with short analysis time (5min). The content of drugs in the formulation was found to be 60mg Pyridoxine and 100mg Doxylamine succinate.The method was validated in terms of sensitivity, accuracy and precision and can be used for the routine determination of Doxylamine succinate and Pyridoxine HCL in pharmaceutical formulations. The above method does not suffer from any interference due to common excipients. Therefore the proposed RP-HPLC method could be successfully applied to estimate commercial pharmaceutical products containing Doxylamine succinate and Pyridoxine HCL.

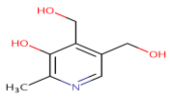
**Keywords:** Symmetry C18, Doxylamine succinate and Pyridoxine, RP-HPLC.

## INTRODUCTION

A combination of Doxylamine with Pyridoxine (B6) is recommended by Gynecologists for pregnant woman who feel discomfort due to Morning sickness. Doxylamine succinate chemically named as dimethyl ({2-[1-phenyl-1-(pyridin-2-yl) ethoxy] ethyl}) amine it is Histamine H1 antagonist with pronounced sedative properties. It is used in allergies and as an antitussive, antiemetic, hypnotic and formerly used in Parkinsonism. It also has substantial sedative and anticholinergic effects. It is a combination of a pain reliever, a cough suppressant and an antihistamine. It is used to treat the aches and pains, cough, fever, headache, runny nose, and sneezing of a cold.



**Doxylamine**



**Pyridoxine**

Pyridoxine is 4,5-bis (hydroxymethyl)-2-methylpyridin-3-ol known as Vitamin B6 which is converted to biologically active coenzyme Pyridoxal 5-phosphate involved in synthesis of amino acids, glycogen, neurotransmitters (serotonin, norepinephrine), sphingolipids, aminolevulinic acid, nucleic acids, hemoglobin, sphingomyelin and other sphingolipids, neurotransmitters such as serotonin, dopamine, norepinephrine and gamma-aminobutyric acid (GABA).

Corresponding Author:- **S.Selvakumar** Email: drselvakumar69@gmail.com

The few analytical spectroscopic [1- 4], and HPLC methods [5-9] appeared in the literature for the determination of simultaneous estimation of Doxylamine succinate and Pyridoxine HCL. In view of the need for a suitable RP-HPLC method for routine analysis of Doxylamine succinate and Pyridoxine HCL in formulation, an attempt was made to develop simple, selective, specificity precise and accurate analytical method for estimation of Doxylamine succinate and Pyridoxine HCL and to find out its applicability and determination in formulation.

## MATERIALS AND INSTRUMENTS USED

**Instruments used:**

Equipment : High performance liquid chromatography equipped with Auto Sampler Software: Empower 2 (WATERS), Column:Symmetry C18 (4.6 x 150mm, 5µm, Make:Waters) Detector : PDA detector, Elico pH meter, LABINDIA 3000 – Double beam UV-VISIBLE spectrophotometer, Vacuum filter pump, Digital balance.

**Reagents and Standard:**

Water HPLC Grade, Methanol HPLC Grade, Acetonitrile HPLC Grade, Orthophosphoric Acid, Potassium dihydrogen Ortho phosphate, Pyridoxine HCL & Doxylamine succinate working Standards, Pyridoxine HC L& Doxylamine succinate Tablets

## METHADOLOGY

**Selection of mobile phase:**

Pure drug of Doxylamine succinate and Pyridoxine HCL mixed standard stock solution (10µg/mL of Doxylamine succinate and 10µg/mL of Pyridoxine HCL) were taken and 10µL sample was injected in to RP-HPLC system and run in different solvent systems.

Different mobile phase compositions of pH 3.5 phosphate buffer: ACN (30:70) in order to determine the best conditions for the effective separation and elution of the analytes.

The mobile phase consisting of pH 3.5 buffer: ACN (30:70) was selected.

**Preparation of Phosphate buffer:($P^H$:3.5)**

Weighed 7.0 grams of Potassium Di hydrogen Ortho Phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. PH 3.5 adjusted with Orthophosphoric acid.

**Preparation of mobile phase:**

Mixture of above Buffer 250 mL (25%),750 mL of Acetonitrile HPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter under vacuum filtration.

**Selection of Flow rate:**

A chromatogram was run with the optimized mobile phase, and some different flow rates of 0.8mL/min, 1mL/min, 1.2mL/min and were tried. The best retention time and separation was obtained at 1.0mL/min, so the flow rate of 1.0 mL/min has been selected.

**Preparation of the Pyridoxine HCL& Doxylamine succinate**

**Standard Solution:**

Accurately weigh and transfer 12 mg of Pyridoxine HCL and 10mg of Doxylamine succinate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent mobile phase.

**Selection of analytical wavelength;**

By appropriate dilutions of the standard stock solutions with methanol, various concentrations of Doxylamine succinate and Pyridoxine HCL were prepared separately and their overlain spectra was obtained using the double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. From the overlain spectra, it was observed that Doxylamine succinate and Pyridoxine HCL exhibited strong absorbance at about 254 nm (it is the coinciding maximum absorbance where the two drugs can be detected sufficiently enough for quantitative evaluation) which was selected as the analytical wavelength for further analysis.

**Preparation of the Pyridoxine HCL& Doxylamine succinate**

**Sample Solution:**

Accurately weighed and transferred equivalent to 256.9 mg of Pyridoxine HCL and Doxylamine succinate sample into a 100mL clean dry volumetric flask add about 70mL of Diluent (mobile phase) and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.6ml of Pyridoxine HCL and Doxylamine succinate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent (mobile phase).

## ANALYTICAL VALIDATION OF THE PROPOSED METHOD

**System suitability studies:**

System suitability studies were carried out as specified in the United States Pharmacopoeia (USP). These parameters include column efficiency, resolution, capacity factor, tailing factor and HETP were calculated in present study.

**Specificity:**

**Preparation of Level – I**

**(12ppm of Pyridoxine HCL&20ppm of Doxylamine succinate):**
0.1&0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent (mobile phase).

**Preparation of Level – II**
**(24ppm of Pyridoxine HCL&40ppm of Doxylamine succinate):**
0.2&0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. (mobile phase).

**Preparation of Level – III**
**(36ppm of Pyridoxine HCL&60ppm of Doxylamine succinate):**
0.3&0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. (mobile phase).

**Preparation of Level – IV**
**(48ppm of Pyridoxine HCL&80ppm of Doxylamine succinate):**
0.4&0.8ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. (mobile phase).

**Preparation of Level – V**
**(60ppm of Pyridoxine HCL&100ppm of Doxylamine succinate)**
0.5&1.0ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent. (mobile phase).

**Procedure:** Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

**Linearity Results: (for Pyridoxine HCL)**
**PRECISION STUDIES:**
Expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions

**Procedure:**
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Acceptance Criteria:**
The % RSD for the area of five standard injections results should not be more than 2%.

**INTERMEDIATE PRECISION/ RUGGEDNESS:**
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

**Procedure:**
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
**Acceptance Criteria:** The % RSD for the area of five standard injections results should not be more than 2%.

**ACCURACY STUDIES:**
**Preparation Sample solutions:**
**For preparation of 50% solution (With respect to target Assay concentration):**
Accurately weigh and transfer 6mg of Pyridoxine HCL and 5mg of Doxylamine succinate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
(Stock Solution).
Further pipette 0.3&0.6ml of Pyridoxine HCL& Doxylamine succinate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with 37iluents.

**For preparation of 100% solution: (With respect to target Assay concentration)**
Accurately weigh and transfer 12 mg of Pyridoxine HCL and 10mg of Doxylamine succinate working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3&0.6ml of Pyridoxine HCL& Doxylamine succinate **of** the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

**For preparation of 150% solution: (With respect to target Assay concentration)**
Accurately weigh and transfer 18mg of Pyridoxine HCL and 15mg of Doxylamine succinate working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
(Stock solution).
Further pipette 0.3&0.6ml of Pyridoxine HCL& Doxylamine succinate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [5-8].

**Procedure:**

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Pyridoxine HCL& Doxylamine succinate and calculate the individual recovery and mean recovery values.

**Acceptance Criteria:**

- The % Recovery for each level should be between 98.0 to 102.0%.

**The accuracy results for Doxylamine succinate**

**Acceptance Criteria:**

- The % Recovery for each level should be between 98.0 to 102.0%

**ROBUSTNESS:**

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

**a). The flow rate was varied at 0.8 ml/min to 1.2ml/min.**

Standard solution 36ppm of Pyridoxine HCL & 60ppm of Doxylamine succinate was prepared and analysed using the varied flow rates along with method flow rate.

**The results are summarized:** On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

**System suitability results for Pyridoxine HCL:**

**b). The Organic composition in the Mobile phase was varied from 70% to 80%.**

Standard solution 36 µg/ml of Pyridoxine HCL & 60µg/ml of Doxylamine succinate was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method

**The results are summarized:**

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10

System suitability results for Pyridoxine HCL:

**LIMIT OF DETECTION: (for Pyridoxine HCL)**

**Preparation of 36µg/ml solution:**

Accurately weigh and transfer 12 mg of Pyridoxine HCL working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of 0.03µg/ml solution):**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents Pipette 0.9mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

**Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 163/55 = 2.96

**Acceptance Criteria:**

- S/N Ratio value shall be 3 for LOD solution.

**LIMIT OF DETECTION: (for Doxylamine succinate)**

**Preparation of 60µg/ml solution:**

Accurately weigh and transfer 10mg of Doxylamine succinate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. mark with diluent [9].

**Preparation of 0.05µg/ml solution):**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.9mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

**Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N =166/55 =3.01

**Acceptance Criteria:**

- S/N Ratio value shall be 3 for LOD solution.

**LIMIT OF QUANTIFICATION: (for Pyridoxine HCL)**

- **Preparation of 30µg/ml solution:**
- Accurately weigh and transfer 12 mg of Pyridoxine HCL working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
- (Stock solution)
- Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

- **Preparation of 0.09µg/ml solution):**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 2.7 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

**Calculation of S/N Ratio:**
Average Baseline Noise obtained from Blank: 55 µV
Signal Obtained from LOQ solution: 543µV
S/N = 543/55 = 9.87

**Acceptance Criteria:**
- S/N Ratio value shall be 10 for LOQ solution.

**LIMIT OF QUANTIFICATION: (for Doxylamine succinate)**

**Preparation of 60µg/ml solution:**

Accurately weigh and transfer 10mg of Doxylamine succinate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of 0.18µg/ml solution):**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.3mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

**Calculation of S/N Ratio:**
Average Baseline Noise obtained from Blank
Signal Obtained from LOQ solution
S/N = 548/55 = 9.96

**Acceptance criteria:**
- S/N Ratio value shall be 10 for LOQ solution.

**Linearity Results: (for Pyridoxine HCL)**
**Table 1. Linearity Results of Pyridoxine HCL**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 12ppm | 374052 |
| 2 | II | 24ppm | 682802 |
| 3 | III | 36ppm | 1012619 |
| 4 | IV | 48ppm | 1324938 |
| 5 | V | 60ppm | 1708316 |
| Correlation Coefficient | | | 0.999 |

**Linearity Results: (for Doxylamine succinate)**
**Table 2. Linearity Results of Doxylamine succinate.**

| Injection | Area |
|---|---|
| Injection-1 | 1010585 |
| Injection-2 | 1011075 |
| Injection-3 | 1011924 |
| Injection-4 | 1014299 |
| Injection-5 | 1022159 |
| Injection-6 | 1014008 |
| **Average** | 1014008.4 |
| **Standard Deviation** | 4774.6 |
| **%RSD** | 0.5 |

**Table 3. The results are summarized Pyridoxine HCL**
**The results are summarized Doxylamine succinate**

| Injection | Area |
|---|---|
| Injection-1 | 1513391 |
| Injection-2 | 1513391 |
| Injection-3 | 1526673 |
| Injection-4 | 1560819 |
| Injection-5 | 1560819 |
| Injection-6 | 1535018 |
| **Average** | 1535018.7 |
| **Standard Deviation** | 24168.8 |
| **%RSD** | 1.6 |

**Table 4. Precision Results of Doxylamine succinate**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 20ppm | 545062 |
| 2 | II | 40ppm | 1052605 |
| 3 | III | 60ppm | 1515308 |
| 4 | IV | 80ppm | 2050501 |
| 5 | V | 100ppm | 2635141 |
| Correlation Coefficient | | | 0.999 |

**Acceptance Criteria:** Correlation coefficient should be not less than 0.999.

**Table 5. The accuracy results for Pyridoxine HCL**

| % Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 605652.5 | 6 | 5.8 | 98.1% | 100.0% |
| 100% | 1246314 | 12 | 12.1 | 101.0% | |
| 150% | 1869868 | 18 | 18.1 | 101.0% | |

**Table 6. The accuracy results for Doxylamine succinate**

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 774787.7 | 5 | 5.0 | 101.3% | 100.3% |
| 100% | 1537580 | 10 | 10.0 | 100.3% | |
| 150% | 2285575 | 15 | 14.9 | 99.4% | |

**Table 7. System suitability results for Pyridoxine HCL:**

| S.No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.8 | 4479.0 | 1.3 |
| 2 | 1.0 | 4750.0 | 1.2 |
| 3 | 1.2 | 4099.0 | 1.2 |

**Table 8. System suitability results for Doxylamine succinate:**

| S.No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.8 | 3086.0 | 1.1 |
| 2 | 1.0 | 3744.0 | 1.2 |
| 3 | 1.2 | 3072.0 | 1.1 |

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

**Table 9. System suitability results for Pyridoxine HCL**

| S.No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 2028.0 | 0.9 |
| 2 | *Actual | 4750.0 | 1.2 |
| 3 | 10% more | 2013.0 | 1.0 |

**Table .10 System suitability results for Doxylamine succinate**

| S.No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 3035.0 | 1.0 |
| 2 | *Actual | 3744.0 | 1.2 |
| 3 | 10% more | 3002.0 | 1.0 |

***** Results for actual Mobile phase composition (25:75Buffer: Acetonitrile) have been considered from Accuracy standard.

**Table. 11 Showing parameters of doxylamine succinate & pyridoxine**

| Parameters | Doxylamine succinate | Pyridoxine HCL |
|---|---|---|
| Accuracy | % Recovery =100.0% | % Recovery =100.3 |
| precision | % RSD =1.6% | % RSD =0.5% |
| Id precision | % RSD = 0.9% | % RSD = 0.7% |
| Linearity | $R^2$ =0.999 | $R^2$ =0.999 |
| Range | 12-60 | 20-100 |
| Limit of detection | 0.2 µg/ml | 1.0 µg/ml |
| Limit of quantitation | 0.5 µg/ml | 1.2 µg/ml |

**Fig.1 Showing overlap Doxylamine succinate and Pyridoxine HCL**

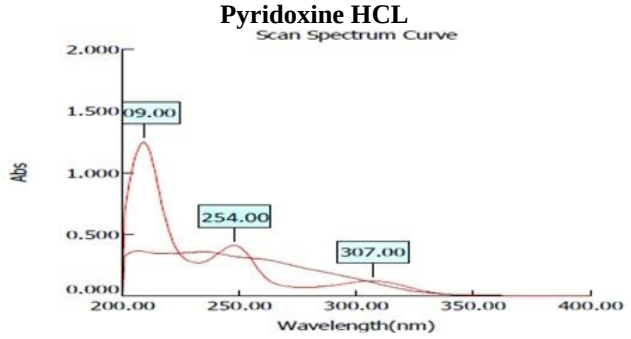



**Fig 2. Pyridoxine HCL Calibration curve graph**
Correlation Coefficient is 0.999.

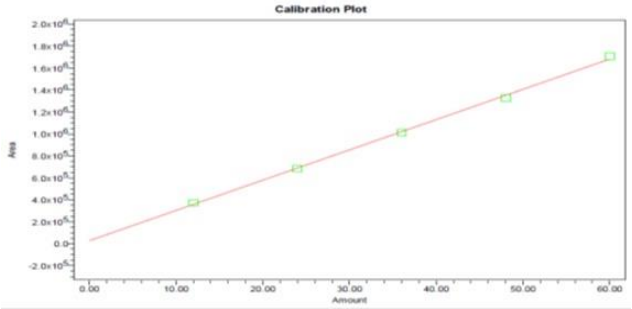



**Fig.3 Doxylamine succinate Calibration curve graph**

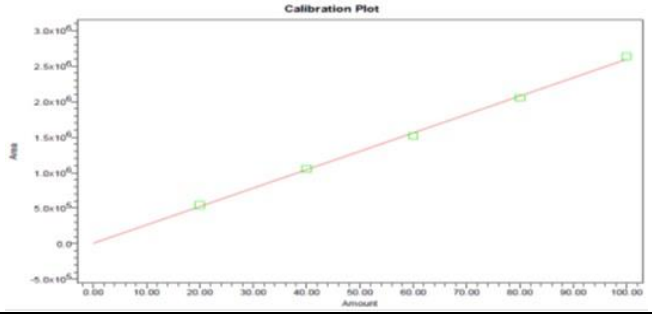



**Fig.4 Chromatogram Linearity level-1**

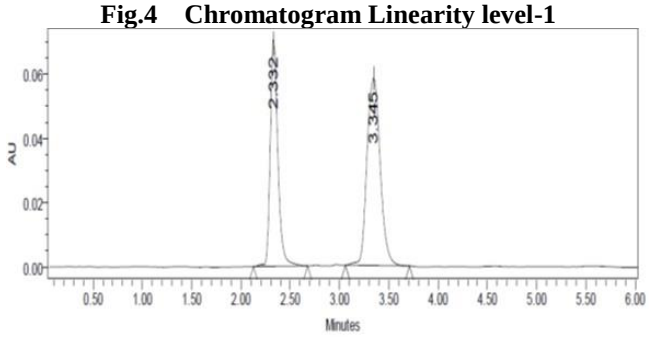



**Fig.5 Chromatogram Linearity level-2**

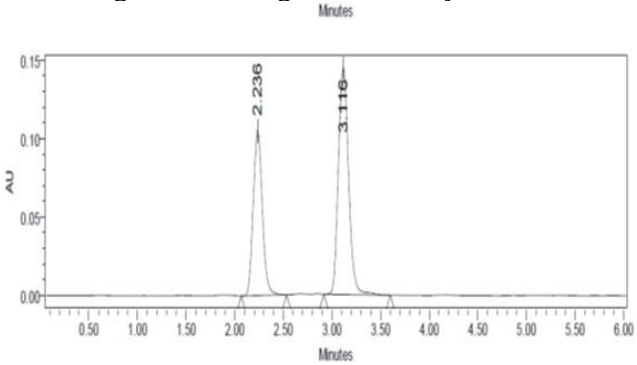



**Fig.6. Chromatogram Linearity level-3**

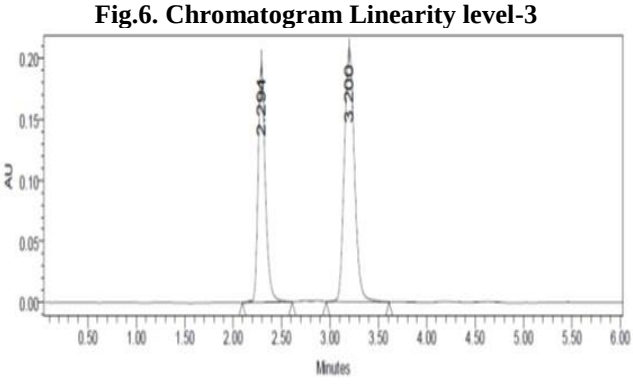


**Fig.7 Chromatogram Linearity level-4**

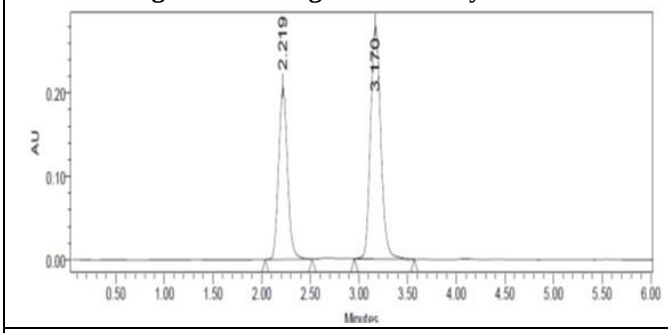
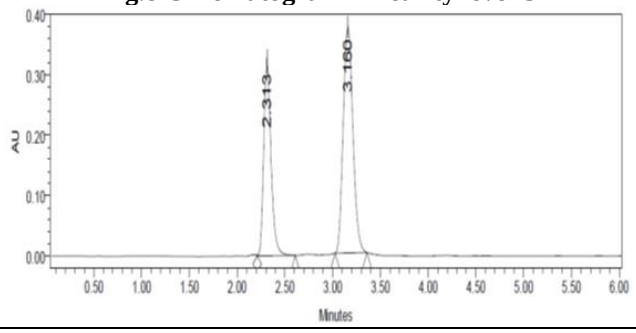
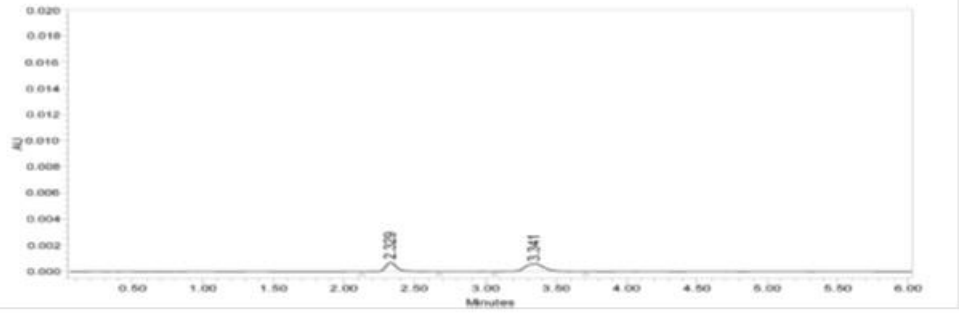



**Fig.8 Chromatogram Linearity level-5**




**Limit of quantification of Doxylamine succinate and Pyridoxine HCL chromatogram: Fig 9**




## RESULT AND DISCUSSION

The suitability of the system was studied by the values obtained for Theoretical plate, Resolution and tailing factor of the chromatogram of standard drugs and presented. The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found.

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out by preparing 3 individual samples with same procedure from the formulation and injecting. The percentage recovery and percentage relative standard deviation of the percentage recovery was calculated and presented in Tables 5 & 6. From the data obtained, added of standard drugs were found to be accurate.

The precision of the method was demonstrated by system and method precision. All solutions were injected into the chromatographic system. The peak area and Standard deviation, precision results presented in Tables 3-4.

The standard drug solution of varying concentration ranging from 12-60µg / ml for Pyridoxine HCL and 20-100µg / ml for Doxylamine succinate. The response factor, slope, intercept and correlation co-efficient were calculated. The slope, intercept, correlation co-efficient were found to be 0.999 for The Pyridoxine 0.999, for Doxylamine succinate calibration curves were plotted using response factor Vs concentration of standard solutions . The calibration graph shows that linear response was obtained over the range of concentration used in the assay procedure. These data demonstrates that the method have adequate sensitivity to the analytes. The range demonstrate that the method is linear outside the limits of expected use.

The robustness of the method was studied by carrying out experiments by changing conditions discussed earlier. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters system suitability studies shown in Table 7-10 and hence developed method is said to be robust and ruggedness.

## SUMMARY

Methodology consists of the general principle in HPLC instrumentation protocol for method development and the detail about the proposed RP-HPLC method on the drug is included. Where the RP-HPLC method in which determination of Doxylamine succinate and Pyridoxine HCL was carried out on a Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters) using a mobile phase consisting of pH 3.5 phosphate buffer: Acetonitrile (30:70). The mobile phase was pumped at a rate of 1.0 ml/min and the detection was carried out at 254nm. The retention time of Doxylamine succinate and Pyridoxine HCL was found to be 2.162 and 3.305 min respectively and linearity was in the range of 12-60µg / ml for Pyridoxine HCL and 20-100µg / ml for Doxylamine succinate.

The results obtained in newer RP-HPLC method for determination of Doxylamine succinate and Pyridoxine HCL are tabulated and also discussed about the developed RP-HPLC method. The proposed method is simple cost effective and gives reliable assay results with short analysis time (5min). The content of drugs in the formulation was

found to be 60mg Pyridoxine and 100mg Doxylamine succinate.

The method was validated in terms of sensitivity, accuracy and precision and can be used for the routine determination of Doxylamine succinate and Pyridoxine HCL in pharmaceutical formulations. The above method does not suffer from any interference due to common excipients. Therefore the proposed RP-HPLC method could be successfully applied to estimate commercial pharmaceutical products containing Doxylamine succinate and Pyridoxine HCL.

## CONCLUSION

The results indicating that the proposed methods are precise, accurate, specific and simple. These methods were developed and validated according to the ICH guidelines. So the developed methods can be easily applied for routine analysis.

It is clear from the present study that the RP-HPLC method for the determination of Doxylamine succinate and Pyridoxine HCL is simple, accurate, specific and precise. This method was validated statistically. The results of recovery studies were in good agreement with the respective label claim of the formulation. Thus the method is less time consuming and can be employed for routine batch analysis of Doxylamine succinate and Pyridoxine HCL.

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