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# A New Rp-Hplc Method Develop A New Rp-Hplc Method Development And Validation For Simultaneous Estimation Of Pyridoxine Hydrochloride And Doxylamine Succinate In Bulk Drug And Pharmaceutical Tablet Dosage Form.

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***Abstract:*** *The Present work was to develop a simple, fast, accurate, precise, reproducible, reverse phase high performance liquid chromatographic method for simultaneous estimation of pyridoxine hydrochloride and doxylamine succinate in pharmaceutical tablet dosage form marketed as doxinate. Chromatographic separation was done using Inertsil ODS RP C18 column having dimension of 4.6×250mm having particle size of 5µm, with mobile phase consisting of phosphate buffer pH 3 ±0.02 pH adjusted with ortho phosphoric acid and acetonitril (50:50 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 263nm. The retention times of pyridoxine hydrochloride and doxylamine succinate was found to be 2.35 and 4.80min. The Proposed method has been validated for accuracy, precision, linearity, range and robustness were within the acceptance limit according to ICH guidelines. Linearity for pyridoxine hydrochloride and doxylamine succinate was found in range of 25µg-150µg and correlation coefficient was found to be 0.999 and 0.999, %RSD for method precision was found to be 0.76, 0.82 and for system precision was 0.80 and 0.71 respectively, % mean recovery for pyridoxine hydrochloride and doxylamine succinate was found to be 99.18% to 99.48%. The method was found to be robust even by change in the mobile phase ±5% and in less flow condition. The developed method can be successfully employed for the routine analysis of pyridoxine hydrochloride and doxylamine succinate in API and Pharmaceutical dosage forms.*

***Keywords:*** *Pyridoxine hydrochloride and Doxylamine succinate, RP-HPLC, Method development, Method validation.*

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## I. Introduction

Pyridoxine hydrochloride is 3, 4-Pyridinedimethanol 5-hydroxy-6-methyl- hydrochloride and its chemical formula $C_8H_{11}NO_3 \cdot HCl$ Molecular weight : 205.64 Melting point **:** 214-215$^0$C.It is crystalline in nature and soluble in water and having pKa 5.0 and 9.0 and it's a vitamin nutritional supplement .Doxylamine Succinate is chemically **:** 1-(Isopropylamino)-3-(p-(2-methoxyethyl)phenoxy)- Ethananamine Chemical formula $C_{17}H_{22}N_2O \cdot C_4H_6O_4$Molecular weight : 388.4 pKa **:** 5.8 and 9.3.It is white or creamy powder and soluble in water and alcohol H1 Histamine receptor antagonist. Antihistaminic; sedative; hypnotic.There are several methods have been reported but only two methods were reported in Drug formulations .The aim of this present study is to develop a single method for the Pyridoxine HCl and Doxylamine succinate in a respective dosage form.

## II. Experimental

**Materials and Methods:**

**Selection of wavelength** 10mg of doxylamine succinate and pyridoxine hydrochloride was dissolved in mobile phase. The solution was scanned from 200 -400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for doxylamine succinate and pyridoxine hydrochloride. The isobestic point was taken as detection wavelength and shown in Fig No 01.

**Preparation of Potassium di hydrogen ortho phosphate (0.01N)**

An accurately weighed quantity of 1.3609g of potassium di hydrogen ortho phosphate was dissolved in 1000ml of hplc water and sonicated for 10min for proper dissolution and adjusted to the pH 3 with Ortho phosporic acid. The resulting solution was filtered.

**Preparation of mobile phase**

An accurately measured quantity of 500ml (50%) of potassium di hydrogen ortho phosphate buffer and 500ml (50%) of acetonitril were mixed and sonicated for 5min in ultrasonic water bath. The solution was filtered through 0.22µ filter under vacuum filter filtration.

**Diluent preparation**

Mobile phase was used as the diluent.

**Preparation of the individual doxylamine succinate standard preparation**

An accurately weighed quantity of 10mg of standard Doxylamine succinate was dissolved in 10ml of mobile phase (Phosphate buffer: Acetonitrile in the ratio of 50:50) to get the concentration of 100µg/ml and sonicated for 5min for proper dissolution.

**Preparation of the individual pyridoxine hydrochloride standard preparation**

An accurately weighed quantity of 10mg of standard Pyridoxine hydrochloride was dissolved in 10ml of mobile phase (Phosphate buffer: Acetonitrile in the ratio of 50:50) to get the concentration of 100µg/ml and sonicated for 5min for proper dissolution.

**Preparation of the doxylamine succinate & pyridoxine hydrochloride standard & sample solution**

**Sample solution preparation**

10mg of sample was accurately weighed and dissolved in 10ml of mobile phase to get 100µg/ml concentration and the sample solution sonicated for 5min for proper dissolution. Sample and standard solutions were injected into to the chromatographic system separately. Equal volume of blank (mobile phase) solution was injected into the system. The chromatograms were recorded for sample and standard injections eliminating the peak area of blank.

**Standard solution preparation**

An accurately weighed quantity of 10mg of doxylamine succinate and 10mg of pyridoxine hydrochloride were transferred into 10ml volumetric flask. 8ml of diluent added to the volumetric flask and sonicated it to dissolve completely and volume was adjusted with the same solvent up to the mark.

**Procedure**

20µL of the blank, standard and sample was injected into the chromatographic system and areas for the doxylamine succinate and pyridoxine hydrochloride from the peaks were used for calculating the % assay by using the formulae . The peak area and chromatographs are reported in Fig. No.2.

**Specificity**

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

**Linearity**

**Preparation of stock solution** An accurately weighed quantity of 10mg of doxylamine succinate and 10mg of pyridoxine hydrochloride were transferred into 10ml volumetric flask. 8ml of diluent added to the volumetric flask and sonicated it to dissolve completely and volume was adjusted with the same solvent up to the mark.

**Procedure**

Each level was injected into the chromatographic system and peak area was measured. A graph was plotted against area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated. Results are tabulated in Table.No.1. Calibration graph for pyridoxine hydrochloride and doxylamine succinate are shown in Fig.No.3.

**Range**

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 25µg-125µg of doxylamine succinate and pyridoxine hydrochloride.

**Accuracy**

**Preparation of standard stock solution**

An accurately weighed quantity of 1mg of 1mg of pyridoxine hydrochloride and doxylamine succinate were transferred into 10ml volumetric flask. 8ml of diluent added to the volumetric flask and sonicated it to dissolve completely and volume was adjusted with the same solvent up to the mark.

**Procedure**

The standard solutions of accuracy-50%, 100% and 150% was injected into chromatographic system. Calculate the amount found and amount added for doxylamine succinate and pyridoxine hydrochloride and calculate the individual % recovery and mean % recovery values. The chromatograms are shown in Fig.No.4, and results are tabulated in Table.2.

**Precision**

**Preparation of stock solution**

An accurately weighed quantity of 1mg of doxylamine succinate and 1mg of pyridoxine hydrochloride were transferred into 10ml volumetric flask. 8ml of diluent added to the volumetric flask and sonicated it to dissolve completely and volume was adjusted with the same solvent up to the mark.

**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 five replicate injections was found to be within the specified limits.

**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.

**Preparation of stock solution**

An accurately weighed quantity of 10mg of doxylamine succinate and 10mg of pyridoxine hydrochloride were transferred into 10ml volumetric flask. 8ml of diluent added to the volumetric flask and sonicated it to dissolve completely and volume was adjusted with the same solvent up to the mark.

**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 five replicate injections was found to be within the specified limits. \

**Limit of detection (LOD)**

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula.. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

**Limit of quantification**

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

**Robustness**

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.9ml/min to 1.1 ml/min. Standard solution 10µg/ml of Pyridoxine hydrochloride & 10µg/ml of Doxylamine succinate was prepared and analysed using the varied flow rates along with method flow rate.
b) The organic composition in the mobile phase was varied from 45% to 55 % standard solution 10µg/ml of Pyridoxin hydrochloride & 10 µg/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 chromatograms are shown in Fig.No.10. And results are tabulated in Table. No.4

**System suitability**

10mg of Pyridoxine hydrochloride and 10mg of Doxylamine succinate working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and add about 10ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 10ml of Pyridoxine hydrochloride & Doxylamine succintea from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

**Results**

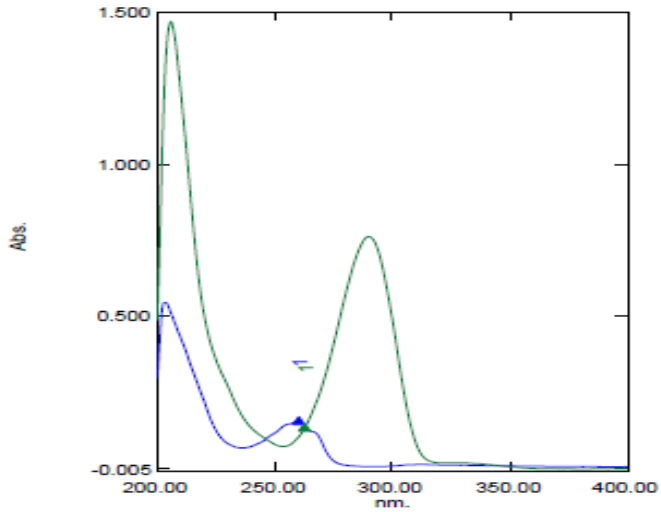


**Fig. No. 1** Spectrum showing overlapping spectrum of DOX & PYR

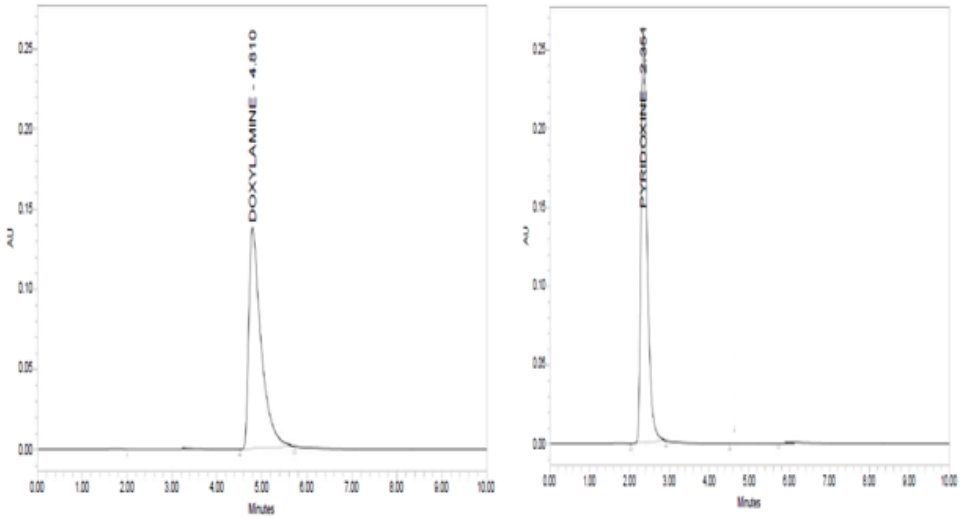


**Fig. No. 2** Chromatogram showing assay injection of PYR and DOX.

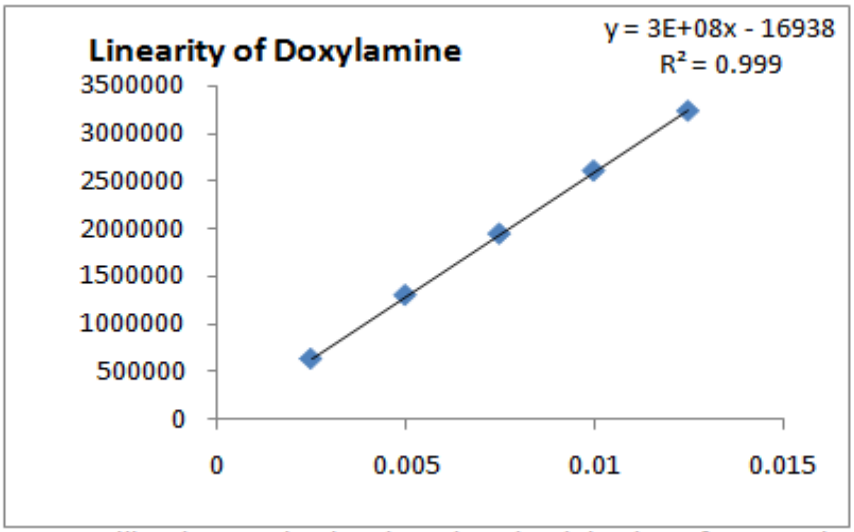


**Fig. No. 3** Calibration graphs showing Linearity injection of PYR and DOX.

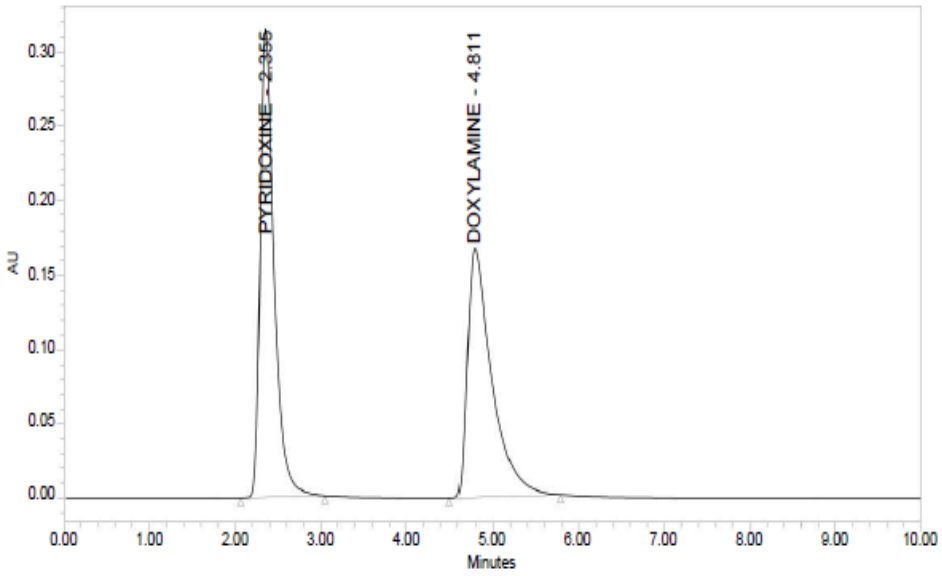


**Fig.No.4** Chromatogram showing accuracy of PYR and DOX.

| S.No | Linearity Level | Concentration | Peak Name | $R_t$ | Area | Height |
|---|---|---|---|---|---|---|
| 1 | I | 2.5µg/ml | Pyridoxine hydrochloride | 2.336 | 692042 | 70517 |
| 2 | II | 5.0µg/ml | Pyridoxine hydrochloride | 2.339 | 1400185 | 143531 |
| 3 | III | 7.5µg/ml | Pyridoxine hydrochloride | 2.344 | 2114074 | 209955 |
| 4 | IV | 10.0µg/ml | Pyridoxine hydrochloride | 2.350 | 2801234 | 262855 |
| 5 | V | 12.5µg/ml | Pyridoxine hydrochloride | 2.354 | 3472210 | 327101 |
| 6 | VI | 15.0µg/ml | Pyridoxine hydrochloride | 2.356 | 3516241 | 315390 |
| Correlation Coefficient | | | | | 0.999 | |

**Table .No.1** Showing linearity results for pyridoxine hydrochloride level 1-6

| S.No | Linearity Level | Concentration | Peak Name | $R_t$ | Area | Height |
|---|---|---|---|---|---|---|
| 1 | I | 2.5µg/ml | Doxylamine succinate | 4.908 | 625674 | 34443 |
| 2 | II | 5.0µg/ml | Doxylamine succinate | 4.850 | 1295362 | 71717 |
| 3 | III | 7.5µg/ml | Doxylamine succinate | 4.817 | 1939653 | 107058 |
| 4 | IV | 10.0µg/ml | Doxylamine succinate | 4.801 | 2602105 | 138479 |
| 5 | V | 12.5µg/ml | Doxylamine succinate | 4.782 | 3232195 | 170954 |
| 6 | VI | 15.0µg/ml | Doxylamine succinate | 4.788 | 3250980 | 168125 |
| Correlation Coefficient | | | | | 0.999 | |

**Table .No.2** Showing linearity results for doxylamine succinate level 1-6

| Conc Microgm/ml | Average Area | | Amount added Microgm/ml | Amount found | | %Recovery | Mean |
|---|---|---|---|---|---|---|---|
| | PYR | DOX | | PYR | DOX | | |
| 50 | 1410548 | 1296543 | 5 | 4.965 | 4.970 | 99.40 | 99.48% |
| 100 | 2815698 | 2611533 | 10 | 9.911 | 9.987 | 99.87 | |
| 150 | 4205786 | 3811501 | 15 | 14.867 | 14.874 | 99.16 | |

**Table .No.**3 Showing Accuracy results for PYR and DOX

| | | | | | |
|---|---|---|---|---|---|
| | **Robustness** | More flow | $R_t$ = 1.94 | $R_t$ = 3.96 | Robust even by change in the flow rate ±0.2ml/min |
| | | Less flow | $R_t$ = 2.997 | $R_t$ = 6.161 | |
| | | More organic | $R_t$ = 2.347 | $R_t$ = 4.580 | Robust even by change in the mobile phase ±5%. |

**Table .No.4** Showing Robustness results for PYR and DOX

## Bibliography


[1]. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
[2]. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
[3]. Andrea Weston and Phyllisr.Brown, HPLC Principle and Practice,1st edition,
[4]. Academic press, (1997), PP 24-37.
[5]. Yuri Kazakevich and Rosario Lobrutto, HPLC For Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 3-6,15-23.
[6]. Francis Rouessac and Annick Rouessac, Chemical Analysis Modern Instrumentation methods and techniques,2nd edition,John Willey & Sons Ltd,(2007),PP 63-71.
[7]. Lloyd R.Snyder, Joseph J.Kirkland, John W.Dolan, Introduction To Modern Liquid Chromatography, 3rd edition,A John Wiley & Sons, Inc., Publication, (2010),PP 65-69.
[8]. David M. Bliesner, Validating Chromatographic Methods, A Practical Guide, A John Wiley & Sons, Inc., Publication, (2006),PP 8-14.
[9]. Introduction to Column (Online),URL:http://amitpatel745.topcities.com/index files/study/column care.pdf
[10]. Detectors used in HPLC (online )URL:http://wiki.answers.com/Q/What detectors are used in HPLC.
[11]. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC Book/Detectors/det uvda.html.
[12]. Detectors (online) ,URL:http://www.dionex.com/enus/webdocs/64842-31644-02 PDA-100.pdf
[13]. Detectors (online),URL:http://www.ncbi.nlm.nih.gov/pubmed/8867705
[14]. Detectors (online),URL:http://www.chem.agilent.com/Library/applications/59643559.pdf
[15]. Detectors (online),URL:http://hplc.chem.shu.edu/new/hplcbook/detector
[16]. Paulo Cesar Pires Rosa, Isabel Cristina Sales Fontes Jardim: Simultaneousdetermination of clobutinol hydrochloride and doxylamine succinate from syrups by RP- HPLC using a new stationary phase containing embedded urea polar groups, volume 48, 2012.


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