International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

R E S E A E C H A R T I C L E

Method Development and Validation of Assay of Pyridoxine Hydrochloride and Doxylamine Succinate

S. Masoom Vali*, G. Somasekhar, Y. Suresh, Haniffa Bee S, K. Suresh

Department of Pharmaceutical Analysis, SAFA College of Pharmacy, Kurnool, Andhra Pradesh, India

A B S T R A C T

A new method was established for simultaneous estimation of Pyridoxine hydrochloride and Doxylamine succinate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Pyridoxine hydrochloride and Doxylamine succinate by using Zorbax Bonus RP (150x4.6) mm, 5µ (Manufacturer: Agilant column, flow rate was 1.0ml/min, detection wave length was 272nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 2.6 mins and 4.9mins. From the developed method it was found that both Pyridoxine HCl and Doxylamine Succinate obey linearity within the concentration range of 2.5-15 µg/ml. From the results of precision and intermediate precision it was found that the % RSD is less than 2, which indicates that the method has good reproducibility. From the results shown in accuracy it was found that the percentage recovery values of formulation from the placebo solution were in between 98.0– 102.0 which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method. From the results of Robustness it was found that a little variation in methods does not affect the intended use. From the results of Forced degradation studies it was found that method is fit to use stability studies.

Keywords: Pyridoxine hydrochloride, Doxylamine succinate, HPLC

A R T I C L E I N F O

CONTENTS

Article History: Received 21 November 2018, **Accepted** 24 December 2017, **Available Online** 27 January 2018

***Corresponding Author**
S. Masoom Vali
Department of Pharmaceutical Analysis,
SAFA College of Pharmacy, Kurnool,
Andhra Pradesh, India
Manuscript ID: IJCPS3517

PAPER-QR CODE

Citation: S. Masoom Vali, *et al.* Method Development and Validation of Assay of Pyridoxine Hydrochloride and Doxylamine Succinate. *Int. J. Chem, Pharm, Sci*., 2018, 6(1): 16-25.

1. Introduction

Analytical methods Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1,2]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

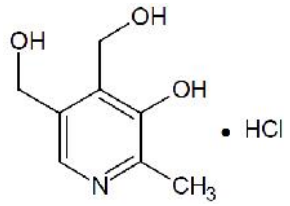

Figure 1: Pyridoxine hydrochloride

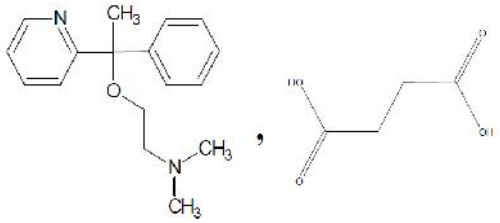

Figure 2: Doxylamine succinate

Chromatography: Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

2. Materials and Methods

Apparatus

The instrument used for the study was WATERS, software: Empower, 2695 separation module, PDA detector.

Mobile phase:

pH 3.5, 0.01M Buffer with n-Butane sulphonic acid sodium salt preparation: 1.36g of potassium di hydrogen phosphate and 1.0g of n-Butane sulphonic acid sodium salt dissolve in 1000mL of water. Adjust pH to 3.50 with ortho phosphoric acid. Mix 550mL of Buffer and 450mL of Methanol, Degas for 5min using sonicator [10]. 0.05N Hydrochloric acid: Dilute 4.3mL of HCl to 1000mL with water.

Diluent: Mix 700mL of 0.05N Hydrochloric acid and 300mL of methanol.

Optimization Chromatographic trials for Simultaneous Estimation of Pyridoxine hydrochloride and Doxylamine succinate by RP- HPLC.

Optimization chromatographic conditions

Column : Zorbax Bonus RP (150x4.6) mm, 5µ (Manufacturer: Agilant)
Column Oven temperature: 30°C
Injection volume : 20µl
Wave length : 272nm
Flow rate : 1.0ml/min
Runtime : 10 min

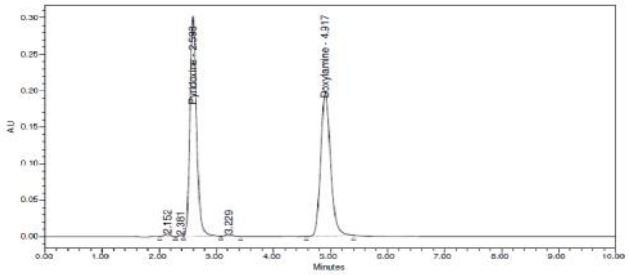

Figure 3: Optimization Chromatogram

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Method Validation

Specificity

Blank Interference: Diluent injected into HPLC to ensure the interferences at retention times of Doxylamine Succinate and Pyridoxine HCl

Placebo Interference: Accurately weighed 105 mg. of Placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent. Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram to ensure the interferences at retention times of Doxylamine Succinate and Pyridoxine HCl Hydrochloride [11].

Linearity: Range from 2.5µg/mL to 15µg/mL i.e 25% to 150% of test concentration (10µg/mL) selected to establish

the linearity as function of analytes concentration Vs response. The Correlation coefficient, Y-Intercept and Slope of regression line calculated.

Standard Stock for Linearity solutions: Accurately weighed 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl HCl taken into clean and dry 100 ml volumetric flask. To this 70mL of diluent added. Sonicated to dissolve volume made up to the mark with diluents [12].

Level-1 (2.5«g/mL): 0.5mL of Standard Stock solution diluted to 20mL with diluent to get each 2.5µg per mL solution mixture of Doxylamine Succinate and Pyridoxine HCl.

Level-2 (5«g/mL): 1mL of Standard Stock solution diluted to 20mL with diluent to get each 5µg per mL solution mixture of Doxylamine Succinate and Pyridoxine HCl.

Level-3 (7.5«g/mL): 1.5mL of Standard Stock solution diluted to 20mL with diluent to get each 7.5µg per mL solution mixture of Doxylamine Succinate and Pyridoxine HCl.

Level-4 (10«g/mL): 2mL of Standard Stock solution diluted to 20mL with diluent to get each 10µg per mL solution mixture of Doxylamine Succinate and Pyridoxine HCl.

Level-5 (12.5«g/mL): 2.5mL of Standard Stock solution diluted to 20mL with diluent to get each 12.5µg per mL solution mixture of Doxylamine Succinate and Pyridoxine HCl.

Level-6 (15«g/mL): 3mL of Standard Stock solution diluted to 20mL with diluent to get each 15µg per mL solution mixture of Doxylamine Succinate and Pyridoxine HCl. Linearity level solutions injected simultaneously into HPLC to record chromatogram.

Accuracy: Range from 8µg/mL to 12µg/mL i.e 80% to 120% of test concentration (10µg/mL) selected to establish the accuracy by recovery studies. Prepared three preparations at each recovery level. The Percentage of recovery at all three levels and %RSD calculated [13].

Standard stock solution preparation

Accurately weighed 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this 70mL of diluent added. Sonicated to dissolve volume made up to the mark with diluents [14].

Standard Solution preparation:

From Standard stock solution preparation 2mL diluted to 20mL with diluent to get concentration of 10µg/mL.

Level-80% (8«g/mL)

Preparation-1: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 8mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram [15].

Preparation-2: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 8mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram [16].

Preparation-3: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 8mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram.

Level-100% (10«g/mL)

Preparation-1: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 10mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram [17].

Preparation-2: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 10mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram [18].

Preparation-3: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 10mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram[19].

Level-120% (12«g/mL)

Preparation-1: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 12mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked.

Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram [20].

Preparation-2: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 12mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram.

Preparation-3: Accurately weighed 105 mg of placebo powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this flask each 12mg of accurately weighed Doxylamine Succinate and Pyridoxine HCl spiked. Then 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram [21].

Precision:

System precision, method precision and intraday precision were performed to ensure the degree of closeness between the responses obtained form series of injections of standard and sample preparations at different time interval of different days. The Percentage Relative Standard Deviation (%RSD) or Coefficient of Variance (CV) and Standard Deviation (SD) were calculated to measure the precision.

System Precision: System precision was carried out on six replicate injections of standard solution.

Standard stock solution preparation

Accurately weighed 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this 70mL of diluent added. Sonicated to dissolve volume made up to the mark with diluents [22].

Standard solution preparation: From Standard stock solution 2mL diluted to 20mL with diluent to get concentration of 10µg/mL**.** Six Successive Injections of same Standard solution injected in to HPLC to measure the system precision.

Method Precision: Method precision was carried out on six sample preparations on calculating Assay values.

Sample solution preparation

Accurately weighed 125 mg. of tablet powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask and 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent. Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram. The other five replicate sample solution preparations were prepared same as above sample solution preparation [23]. Six Successive Injections each from six sample solutions injected in to HPLC to measure the method precision.

Intraday Precision: Intraday precision was carried out on measuring the %RSD value of on the day system precision responses along with previous day system precision responses.

Standard stock solution preparation

Accurately weighed 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this 70mL of diluent added. Sonicated to dissolve volume made up to the mark with diluent.

Standard solution preparation:

From Standard stock solution 2mL diluted to 20mL with diluent to get concentration of 10µg/mL. Six Successive Injections of same Standard solution injected in to HPLC to measure the system precision. The %RSD of twelve responses of day 1 system precision and day 2 system precision was calculate in order to measure the Intraday precision.

Intermediate Precision

Also known as Reproducibility or Ruggedness was carried out by other analyst by calculating Assay values on six sample preparations.

Sample solution preparation

Accurately weighed 125 mg. of tablet powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask and 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent. Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter. Solution injected into HPLC to record chromatogram. The other five replicate sample solution preparations were prepared same as above sample solution preparation. Six Successive Injections each from six sample solutions injected in to HPLC to measure the method precision. The %RSD of twelve Assay values of Analyst I on method precision and Analyst II on method precision was calculate in order to measure the Intermediate precision.

Filter validation: 0.45u PVDF filter was validated using filtered Standard and Sample preparations against unfiltered Standard and centrifuged sample by calculating similarity factor.

$$\text{Similarity factor} = \frac{\text{Area of Filtered Standard or sample}}{\text{Area of Centrifuged or unfiltered Standard or sample}}$$

Standard stock solution preparation

Accurately weighed 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this 70mL of diluent added. Sonicated to dissolve volume made up to the mark with diluent.

Standard solution preparation (Unfiltered):

From Standard stock solution 2mL diluted to 20mL with diluent to get concentration of 10µg/mL and solution injected into HPLC to record chromatogram

Standard solution preparation (Filtered):
Standard solution was filtered through 0.45u PVDF syringe filter and filtered solution injected into HPLC to record chromatogram.

Sample solution preparation (Centrifuged)
Accurately weighed 125 mg. of tablet powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask and 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent. Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent and solution injected into HPLC to record chromatogram

Sample solution preparation (Filtered)
Accurately weighed 125 mg. of tablet powder i.e equivalent to 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask and 70mL of diluent added. Sonicated to for 15min and volume made up to the mark with diluent. Centrifuged solution at 4000rpm for 5min, then 2mL of supernatant solution diluted to 20mL with diluent. Filtered solution through 0.45u PVDF Syringe filter and filtered solution injected into HPLC to record chromatogram.

Robustness

Variation in mobile phase composition
Robustness of method with respect variation in mobile phase composition was carried out and all the system suitability parameters were monitored to ensure adherence to proposed acceptance criteria.
Positive variation: (Buffer: methanol) 50:50
Negative variation: (Buffer: methanol) 60:40

Variation in pH of mobile phase: Robustness of method with respect variation in pH mobile phase was carried out and all the system suitability parameters were monitored to ensure adherence to proposed acceptance criteria.
Positive variation: pH3.7 Buffer
Negative variation: pH 3.3Buffer

Variation in Column oven temperature: Robustness of method with respect variation in Column oven temperature was carried out and all the system suitability parameters were monitored to ensure adherence to proposed acceptance criteria.
Positive variation: 35°C
Negative variation: 25°C

Variation in Flow rate: Robustness of method with respect variation in Column oven temperature was carried out and all the system suitability parameters were monitored to ensure adherence to proposed acceptance criteria.
Positive variation: 1.2mL/min
Negative variation: 0.8mL/min

Limit of detection: The parameter LOD was determined on the basis of response and slope of the regression equation.

Limit of Quantification: The parameter LOQ was determined on the basis of response and slope of the regression equation.

3. Results and Discussion

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.

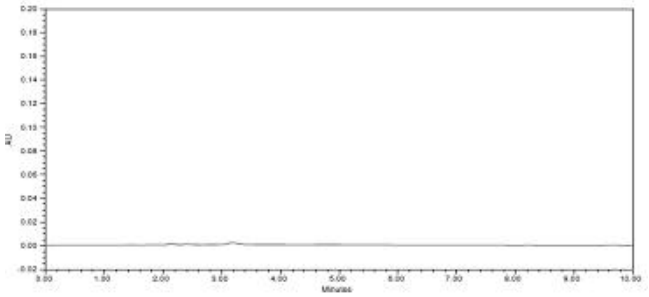

Figure 4: Chromatogram of Blank

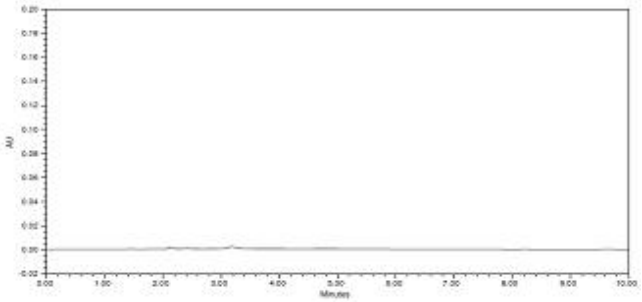

Figure 5: Chromatogram of placebo

2. Linearity: The linearity study was performed for the concentration of 25 ppm to 150ppm for Pyridoxine hcl and 25ppm to 150ppm for Doxylamine succinate and chromatograms are shown below.

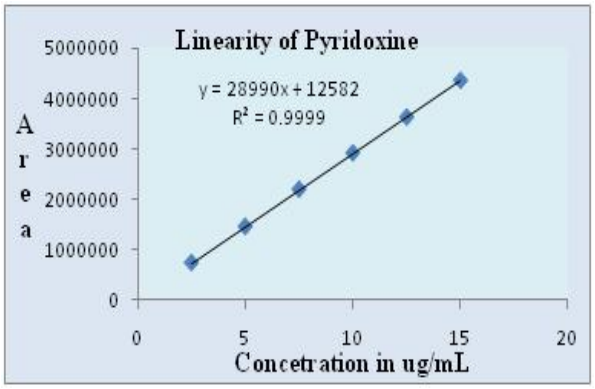

Figure 6: Calibration graph of Pyridoxine hcl

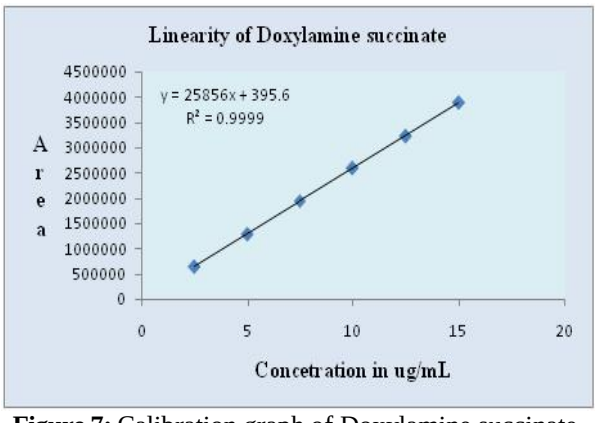

Figure 7: Calibration graph of Doxylamine succinate

Table 1: Linearity Results for Pyridoxine hcl

S.No	% Level	Concentration (mcg)	Area
1	25	2.5	736627
2	50	5	1455245
3	75	7.5	2194495
4	100	10	2918659
5	125	12.5	3630033
6	150	15	4360167
Correlation coefficient (r2)			0.9999
Slope			28990
Y-intercept			12582
SD of Y- intercepts of line			7010

Table 2: Linearity Results for Doxylamine succinate

S.No	% Level	Concentration (mcg)	Area
1	25	2.5	648814
2	50	5	1289459
3	75	7.5	1939595
4	100	10	2593820
5	125	12.5	3221792
6	150	15	3883407
Correlation coefficient (r2)			0.9999
Slope			25856
Y-intercept			395.6
SD of Y- intercepts of line			7292

Table 3: Accuracy results for Pyridoxine hcl

S.No.	Concentration Levels	Percentage Recovery	Mean percentage recovery	Standard deviation	%RSD
1	80%	100.57	100.34	0.22	0.220
2	80%	100.13			
3	80%	100.33			
4	100%	100.40	100.47	0.36	0.358
5	100%	100.86			
6	100%	100.15			
7	120%	100.68	100.70	0.34	0.333
8	120%	100.37			
9	120%	100.04			

Table 4: Accuracy results for Doxylamine succinate

S.No.	Concentration Levels	Percentage Recovery	Mean percentage recovery	Standard deviation	%RSD
1	80%	100.21	100.31	0.08	0.084
2	80%	100.35			
3	80%	100.36			
4	100%	100.80	100.66	0.21	0.207
5	100%	100.76			
6	100%	100.42			
7	120%	100.61	100.41	0.35	0.344
8	120%	100.73			
9	120%	100.08			

Table 5: System Precision

S No	Name	Pyridoxine HCl		Doxylamine Succinate	
		RT	Area	RT	Area
1	S-Precision-1	2.587	2861435	4.889	2543981
2	S-Precision-2	2.585	2867470	4.887	2548094
3	S-Precision-3	2.587	2882141	4.887	2564313
4	S-Precision-4	2.587	2900807	4.888	2564649
5	S-Precision-5	2.586	2901400	4.888	2561875
6	S-Precision-6	2.588	2893936	4.892	2558571
Average		2.587	2884532	4.889	2556914
Standard Deviation		0.00	17135.58	0.00	8797.58
% of RSD		0.04	0.59	0.04	0.34

Table 6: Method Precision

S No	Name	Pyridoxine HCl	Doxylamine Succinate
		%Assay	%Assay
1	M-Precision-1	100.21	100.52
2	M-Precision-2	100.35	100.35
3	M-Precision-3	100.56	100.67
4	M-Precision-4	100.86	100.28
5	M-Precision-5	100.54	100.13
6	M-Precision-6	100.46	100.62
Average		100.50	100.43
Standard Deviation		0.22	0.21
% of RSD		0.219	0.209

Table 7: Intermediate Precision

S No	Name	Pyridoxine HCl	Doxylamine Succinate
		%Assay	%Assay
1	Analyst 1 Precision-1	100.21	100.52
2	Analyst 1 Precision-2	100.35	100.35
3	Analyst 1 Precision-3	100.56	100.67
4	Analyst 1 Precision-4	100.86	100.28
5	Analyst 1 Precision-5	100.54	100.13
6	Analyst 1 Precision-6	100.46	100.62
7	Analyst 2 Precision-1	100.52	100.24
8	Analyst 2 Precision-2	100.28	100.51
9	Analyst 2 Precision-3	100.35	100.23
10	Analyst 2 Precision-4	100.46	100.29
11	Analyst 2 Precision-5	100.25	100.41
12	Analyst 2 Precision-6	100.61	100.73
Average		100.45	100.42
Standard Deviation		0.182	0.193
% of RSD		0.18	0.19

Table 8: Variation in mobile phase composition

System suitability parameter	**Mobile Phase Composition**			**Acceptance criteria**
	50% organic phase	**55% organic phase**	**60% organic phase**	
USP tailing factor of Pyridoxin HCl peak in standard	1.08	1.06	1.02	NMT 2.0
USP tailing factor of Doxylamine Succinate peak in standard	1.13	1.08	1.06	NMT 2.0

Table 9: Variation in pH of mobile phase

System suitability parameter	**pH of mobile phase**			**Acceptance criteria**
	pH3.3	**pH3.5**	**pH3.7**	
USP tailing factor of Pyridoxin HCl peak in standard	1.07	1.06	1.03	NMT 2.0
USP tailing factor of Doxylamine Succinate peak in standard	1.09	1.08	1.02	NMT 2.0

Table 10: Variation in Column oven temperature

System suitability parameter	**Column oven temperature**			**Acceptance criteria**
	25°C	**30°C**	**35°C**	
USP tailing factor of Pyridoxin HCl peak in standard	1.09	1.06	1.02	NMT 2.0

USP tailing factor of Doxylamine Succinate peak in standard	1.14	1.08	1.06	NMT 2.0

Table 11: Variation in Flow rate

System suitability parameter	Flow rate			Acceptance criteria
	0.8mL min	1.0mL/min	1.2mL/min	
USP tailing factor of Pyridoxin HCl peak in standard	1.08	1.06	1.08	NMT 2.0
USP tailing factor of Doxylamine Succinate peak in standard	1.11	1.08	1.09	NMT 2.0

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. A new method was established for simultaneous estimation of Pyridoxine HCl and Doxylamine succinate by RP-HPLC method. From the developed method it was found that both Pyridoxine HCl and Doxylamine Succinate obey linearity within the concentration range of 2.5-15 µg/ml. From the results of precision and intermediate precision it was found that the % RSD is less than 2, which indicates that the method has good reproducibility. From the results shown in accuracy it was found that the percentage recovery values of formulation from the placebo solution were in between 98.0– 102.0 which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method. From the results of Robustness it was found that a little variation in methods does not affect the intended use. From the results of Forced degradation studies it was found that method is fit to use stability studies. The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Pyridoxine HCl and Doxylamine Succinate in pure samples and pharmaceutical formulations.

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