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"DEVELOPMENT OF A SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DOXYLAMINE SUCCINATE AND PYRIDOXINE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM"

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Abstract: A simple, rapid and precise method is developed for the quantitative simultaneous determination of Doxylamine Succinate and Pyridoxine Hydrochloride in bulk and combined pharmaceutical dosage forms. The method was based on UV-Spectrophotometric determination of two drugs, using simultaneous equation method. It involves absorbance measurement at 257 nm (λmax of Doxylamine Succinate) and 324 nm (λmax of Pyridoxine Hydrochloride) in Methanol:water (70:30). For UV Spectrophotometric method, linearity was obtained in concentration range of 5-25 mcg/ml for Doxylamine Succinate and 5-25 mcg/ml for Pyridoxine Hydrochloride respectively, with regression 0.999 and 0.998 for Doxylamine Succinate and Pyridoxine Hydrochloride respectively. Recovery was in the range of 97 -103%; the value of standard deviation and %R.S.D were found to be < 2 %; shows the high precision of the method., in accordance with ICH guidelines. The method has been successively applied to bulk and pharmaceutical formulation and was validated according to ICH guidelines.
KEY WORDS: Doxylamine Succinate, Pyridoxine Hydrochloride, UV Spectrophotometer.

INTRODUCTION

Doxylamine is a first-generation antihistamine. It can be used by itself as a short-term sedative and in combination with other drugs to provide night-time allergy and cold relief. Pyridoxine (vitamin B6) is used to prevent or treat low levels of vitamin B6 in people who do not get enough of the vitamin from their diets. Pyridoxine has been used to prevent or treat a certain nerve disorder (peripheral neuropathy) caused by certain medications (such as isoniazid). Doxylamine incombination with vitamin B_6 (pyridoxine) to prevent morning sickness in pregnant women. Doxylamine is chemically known as (RS)-N, N-dimethyl-2-(1-phenyl-1-pyridin-2-yl-ethoxy)ethanamine, butanedioate (1:1). Doxylamine Succinate [1] is a white or creamy white powder, with molecular formula $C_{17}H_{22}N_2O \cdot C_4H_6O_4$and molecular weight 388.464g/mol. At room temperature it is which is soluble in water, methanol and ethanol. Pyridoxine is chemically known as 4,5-bis(hydroxymethyl)-2-methylpyridin-3-ol hydrochloride. Pyridoxine Hydrochloride[2] is a white powder with

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molecular formula $C_8H_{12}ClNO_3$ and molecular weight 205.638 g/mol. At room temperature it is soluble in water, methanol and ethanol. A detailed survey of the literature for doxylamine succinate and pyridoxine hydrochloride reveals that only a few analytical methods are available for the determination in pure drug and pharmaceutical dosage form using HPLC, Spectrophotometry, LC-MS, Volumetric method, HPTLC densitometric method either in single or combined forms. V. Rajani sekhar*et al* (2014)[3] developed simple and rapid UV spectrophotometric method for simultaneous estimation of doxylamine succinate and pyridoxine hydrochloride in combined dosage form. In this method for both the drugs 260 and 285 nm using methanol as solvent were selected for analysis.This method is costly because it requires more reagent and solvents. So, this method is not suitable for routine analysis of Doxylamine and Pyridoxine. SmitaC.Nayak*et al* (2013) [4] developed a simple, rapid UV Spectrophotometric method for simultaneous estimation of pyridoxine hydrochloride and Doxylamine succinate in bulk and tablet dosage form by simultaneous equation method at 260 and 270nm using 0.1N HCl as solvent. So, it is need to be developed a simple and cost-effective method for the analysis of Doxylamine and Pyridoxine. This paper describes a simple, sensitive and economical method for the simultaneous estimation of Doxylamine and Pyridoxine in its formulation.

Fig. 1: Structure of Doxylamine Succinate

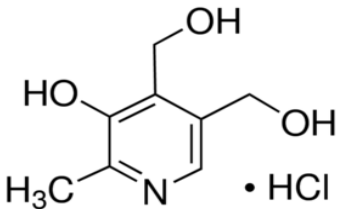

Fig. 2: Structure of Pyridoxine hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents

Doxylamine Succinate and PyridoxineHydrochloridewas obtained as a gift sample from cipla. Doxinate tablets containing Doxylamine Succinate 10 mg and Pyridoxine Hydrochloride 10 mg was procured from Medplus pharmacy, Hyderabad. Distilled water, Methanol (HPLC grade), Water (HPLC grade), were purchased from Merck India Pvt. Limited and LobaChemie India Limited, Mumbai.

INSTRUMENTATION

UV Spectrophotometric method for simultaneous estimation of Doxylamine succinate and pyridoxine hydrochloride

Preparation of stock solution of doxylamine succinate:

An accurately weighed quantity of doxylamine succinate100mg was transferred to 100ml volumetric flask, dissolved in 30ml Methanol, the final volume (70ml) was made with distilled water to obtain standard solution[5] having concentration of 1000µg/m(Stock solution A). 10ml of this solution was transferred to 100ml volumetric flask, volume was made with Water: methanol (70:30) (Stock solution B) It gives 100µg/ml. These stock solutions were used to prepare further dilutions.

Preparation of stock solution of pyridoxine hydrochloride:

An accurately weighed quantity of pyridoxine hydrochloride100mg was transferred to 100ml volumetric flask, dissolved in 30ml Methanol, the final

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volume (70ml) was made with distilled water to obtain standard solution having concentration[6] of 1000µg/m (Stock solution A). 10ml of this solution was transferred to 100ml volumetric flask, volume was made with Water: methanol (70:30) (stock solution B) It gives 100µg/ml. These stock solutions were used to prepare further dilutions.

Selection of Analytical wavelength

Appropriate 10µg/ml dilutions were prepared of doxylamine succinate and pyridoxine hydrochloride from the stock solution B and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained was derivatized for zero order spectroscopy. This zero-order spectrum was selected for the analysis of the drugs. The absorption maximum[7] was found at 259nm for Doxylamine succinate and 324.0nm for pyridoxine Hydrochloride which can be further used for analysis.

Preparation of standard solutions of Doxylamine succinate and pyridoxine hydrochloride

The standard stock solutions of Doxylamine and Pyridoxine were prepared by diluting the stock solution B so as to give a concentration range of 20 µg/ml for Doxylamine and Pyridoxine.

Estimation of tablet dosage form

For the estimation of PYR and DOX in commercial formulations, twenty tablets were weighed and average weight was calculated. The tablets were crushed and tablet powder and powder equivalent to 10 mg of Doxylamine and Pyridoxine were taken and added in 30 ml of methanol and sonicated for 20 min. after sonication the entire solution is filtered through Whatmann filterpaper (No.41) and finally solution was made up to 100 ml with water. 20ml of this stock solution was diluted to 100 ml with methanol:water (70:30)to get concentration equal to 20 µg/ml of doxylamine and 20 µg/ml of Pyridoxine. This solution is scanned in range 200-400 nm taking distilled water as blank. The spectrum obtained was converted to first order derivative spectra, absorbances were noted and concentrations were determined from Simultaneous equation method [8].

Simultaneous Equation method

The two wavelengths selected 257 nm and 324 nm were the wavelengths of maximum absorption of Doxylamine succinate and Pyridoxine HCl. The absorbance and absorptivity's of both drugs were measured at the said wavelengths and the concentrations of the drugs were calculated using the simultaneous equation as follows

$$Cx = (A_2ay_1 - A_1ay_2) / (ax_2ay_1 - ax_1ay_2)$$

$$Cy = (A_1ax_2 - A_2ax_1) / (ax_2ay_1 - ax_1ay_2)$$

C_x = concentration of Doxylamine Succinate

A_1 =absorbance of samples at 257 nm

C_y = concentration of Pyridoxine HCl

A_2= absorbance of samples at 324 nm.

ax_1 is the absorptivity of Doxylamine at 257 nm.

ay_1 is the absorptivity of Doxylamine at 324 nm

ax_2 is the absorptivity of Pyridoxine at 257 nm.

ay_2 is the absorptivity of Pyrdoxine at 324 nm

Validation of proposed method

The developed method was validated according to ICH guidelines [9]in order to determine the linearity, precision, accuracy and ruggedness of the method. For all the parameters %RSD were calculated.

Specificity: Specificity of an analytical method is its ability to measure accurately and specifically the concentration of analyte (s) of interest without interference from other API, diluents, mobile phase & placebo (excipients omitting drug substances).

Procedure: Above mentioned solutions (solvent, sample solution, standard

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solution) were analyzed at 257nm and 324nm. Absorption maxima were recorded and no interferences in blank were reported. UV spectrum of standard and sample solutions were reported and their lamda max were observed.

Acceptance criteria: There should not be any peak in the blank run at the lamda max corroding to standard solutions of doxylamine succinate and pyridoxine hydrochloride.In the sample spectrum, lamda max of sample corresponds to the lamda max of standard solution.

Linearity: The linearity[10] of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity was determined as linear regression with least square method on standard solution. Concentration levels were 5, 10, and 15,20,25 of the claimed analyte concentration, corresponding to the range of about 5-25 mcg/mL

Linearity was demonstrated by preparing the following solution:

Preparation of standard solutions of doxylamine succinate

From the stock solution B of doxylamine succinate following standard solutions was prepared using Water: Methanol (70:30) as solvent.

Linearity level – 1 L_1 Dilute 0.5 ml of stock solution B to 10 ml with solvent (5mcg/ml of doxylamine succinate)

Linearity level – 2 L_2 Dilute 1 ml of stock solution B to 10 ml with solvent (10mcg/ml of doxylamine succinate)

Linearity level – 3 L_3 Dilute 1.5 ml of stock solution B to 10 ml with solvent (15mcg/ml of doxylamine succinate).

Linearity level – 4 L_4 Dilute 2.0 ml of stock solution B to 10 ml with solvent (20mcg/ml of doxylamine succinate).

Linearity level – 5 Dilute 2.5 ml of stock solution B to 10 ml with solvent (25mcg/ml of doxylamine succinate).

Preparation of standard solutions of doxylamine succinate

From the stock solution B of pyridoxine hydrochloride following standard solutions were prepared using water: methanol (70:30) as solvent.

- Linearity level – 1 L_1 Dilute 0.5 ml of stock solution B to 10 ml with solvent (5mcg/ml pyridoxine hydrochloride)
- Linearity level – 2 L_2 Dilute 1 ml of stock solution B to 10 ml with solvent (10mcg/ml of pyridoxine hydrochloride)
- Linearity level – 3 L_3 Dilute 1.5 ml of stock solution B to 10 ml with solvent (15mcg/ml of pyridoxine hydrochloride).
- Linearity level – 4 L_4 Dilute 2.0 ml of stock solution B to 10 ml with solvent (20mcg/ml of pyridoxine hydrochloride)
- Linearity level – 5 Dilute 2.5 ml of stock solution B to 10 ml with solvent (25mcg/ml of pyridoxine hydrochloride)

Procedure: Blank solution was placed in cuvettes zero calibration was done. Linearity level solutions (L_1, L_2, L_3, L_4, L_5 and L_6) were taken in cuvettes and absorbance were measured and chromatograms were recorded.

Accuracy: The accuracy[11] of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure is established across its range. The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100%, 120% in which the amount of marketed formulation (DOXINATE 10/10mg) was kept constant and the amount of pure drug added was varied that is 8mg, 10mg, 12mg for 80%,100% and 120% respectively. The solutions were prepared in triplicates and accuracy was indicated by %recovery. Accuracy

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was demonstrated by preparing following solutions.

S_1 solution (80% of target concentration): Sample formulation at level of 80%, 2.5ml of stock solution A of DOXINATE was dissolved in same solvent (water:methanol-70:30) and made up to 10ml with the same solvent and such that aliquot contain 25mcg/ml. A known quantity of pure drug that is 80mcg was added to 80% level sample solution. These solutions were prepared in triplicates.

S_2 solution (100% of target concentration): Sample formulation at level of 80%, 2.5ml of stock solution A of DOXINATE was dissolved in same solvent (water:methanol-70:30) and made up to 10ml with the same solvent and such that aliquot contain 25mcg/ml. A known quantity of pure drug that is 100mcg was added to 100% level sample solution. These solutions were prepared in triplicates.

S_3 solution (120% of target concentration): Sample formulation at level of 80%, 2.5ml of stock solution A of DOXINATE was dissolved in same solvent (water:methanol-70:30) and made up to 10ml with the same solvent and such that aliquot contain 25mcg/ml. Some known quantities of pure drug that is 120mcg was added to 120% level sample solution. These solutions were prepared in triplicates.

Procedure: Blank solution was placed in cuvettes zero calibration was done. Three sets of sample solutions S_1, S2, S3 prepared were placed in cuvettes and absorbance were determined. The accuracy of this method was determined by measuring %recovery of drug product.

Acceptance criteria: Percentage recovery in all the cases should be between 97.0 and 103.0 %.

Precision: The precision [12] is the parameter that expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple analysis of the same homogenous sample under the prescribed conditions. This experiment was conducted to prove the repeatability of the assay results obtained by this quantification methodology.

System precision

Procedure: Blank solution was placed in cuvettes zero calibration was done2.5 ml of standard solution of doxylamine succinate and pyridoxine hydrochloride were diluted to 10 ml with solvent (25mcg/ml of doxylamine succinate and pyridoxine hydrochloride).

Above standard solution was measured for six times. The %RSD for absorbances were calculated. Calculated the mean and % RSD for the same.

Method precision

Procedure: Blank solution was placed in cuvettes zero calibration was done. 2.5 ml of standard solution of doxylamine succinate and pyridoxine hydrochloride were diluted to 10 ml with solvent (25mcg/ml of doxylamine succinate and pyridoxine hydrochloride). Above standard solution was measured for six times. The %RSD for absorbances were calculated. Calculated the mean and % RSD for the same.

Acceptance criteria: Relative standard deviation (RSD) should not be more than 2.0% Relative standard deviation (RSD) for content of sample in percentage of label claim should not be more than 2.0% in sample solution of DOXINATE10/10mg

Ruggedness: The ruggedness [13] of an analytical method is the degree of reproducibility of test results obtained by the same samples under a variety of conditions, such as different laboratories, different analyst and different instrument etc. To evaluate the ruggedness (also known as intermediate prevision) of the method, standard solutions of doxylamine succinate and pyridoxine hydrochloride

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Formulation Doxinate 10/10	Label claim (10mg)	Concentration (mcg/ml)	Amount found (mcg/ml)
Doxylamine	10	20	19.572
Pyridoxine	10	20	19.322

was measured on different days and by different analyst.

Procedure: Blank solution was placed in cuvettes zero calibration was done. 2.5 ml of standard solution of doxylamine succinate and pyridoxine hydrochloride were diluted to 10 ml with solvent (25mcg/ml of doxylamine succinate and pyridoxine hydrochloride). Above standard solution was measured for six times. The %RSD for absorbances were calculated. Calculated the mean and % RSD for the same.

RESULTS AND DISCUSSION

Simultaneous estimation of drug formulation

UV spectrum of doxylamine succinate and pyridoxine hydrochloride was recorded from which 257nm and 324nm were selected as wavelength. The UV spectrum recorded for doxylamine and pyridoxine was shown in Fig no. 3 and 4

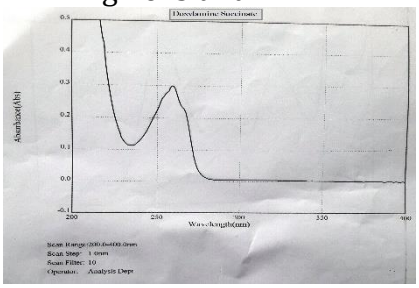

Fig.3 UV Spectrum of doxylamine succinate

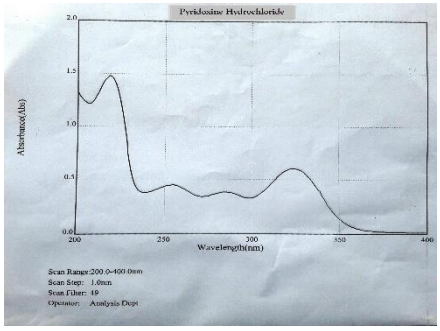

Fig. 4 UV spectrum of Pyridoxine HCl

Table 1: Simultaneous estimation of drug formulation

Specificity: The analyte was assessed in the presence of the components and it was found that there was no interaction with the analyte.

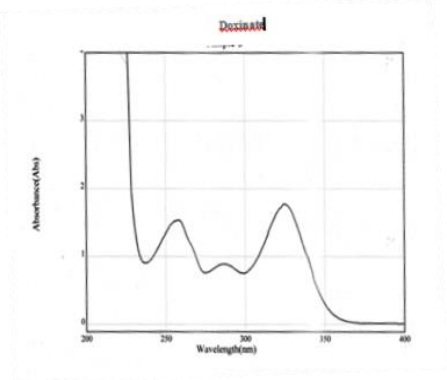

Fig.5 UV Spectrum of Doxinate

Linearity: The linearity was determined as linear regression with least square method on standard solutions. Concentration levels were 5,10,15,20 and 25 of the claimed analyte concentrations to the range of 5-25 mcg/ml. the calibration curve obtained by plotting absorbance versus concentration was found linear in the mentioned concentration range of 5-25mcg/ml for the acceptance of linearity, the correlation coefficient of linearity curve shall not be less than 0.999. the results indicated thatthe method was linear up to the specified concentration range. The linearity results were shown in Table 2 & 3 and linearity graph in Fig. No. 6 & 7.

Table 2: Linearity of Doxylamine succinate working standard.

S.No	Solution	Concentration(mcg/ml)	absorbance
1	L1	5	0.0714
2	L2	10	0.1277
3	L3	15	0.1852
4	L4	20	0.2489
5	L5	25	0.3072

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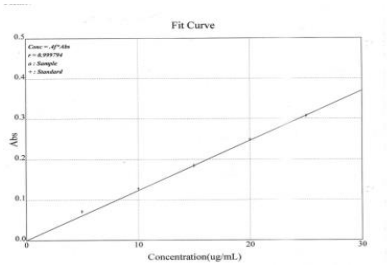

Fig.No.6: Linearity curve of doxylamine succinate

Table 3: Linearity of Pyridoxine Hydrochloride working standard.

S. No	Solution	Concentration(mcg/ml)	absorbance
1	L1	5	0.0872
2	L2	10	0.1595
3	L3	15	0.2428
4	L4	20	0.3320
5	L5	25	0.4120

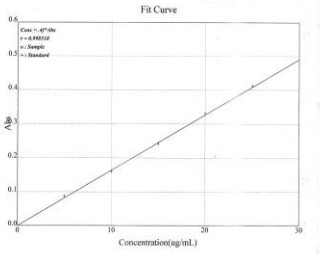

Fig. No. 7 : Linearity curve of Pyridoxine HCl

Precision

The precision of the method was ascertained from determinations absorbance of six replicate injections of standard drug (system precision) and fixed amount of sample drug (method precision). The percentage standard deviations were calculated and presented in the Table 5 and 6. %RSD for percentage assay results of six replicate injections should not be more than 2.0 for acceptance of repeatability. The %RSD (Table 5 and 6) was found to be less than 2.0 hence it concluded that the method was precise and reproducible for the analysis of dorzolamide hydrochloride in the formulation.

Table 4: Results of System Precision of Doxylamine succinate

S. No	Absorbance
1	0.2535
2	0.2761
3	0.2549
4	0.2759
5	0.2411
6	0.2524
AVG	0.2589
SD	0.015
%RSD	0.506

Table 5: Results of System Precision of pyridoxine hydrochloride

S.No	Absorbance
1	0.7109
2	0.7084
3	0.7103
4	0.7084
5	0.7072
6	0.7062
AVG	0.7086
SD	0.017
%RSD	0.506

Table 6: Results of Method Precision of Doxylamine succinate and Pyridoxine HCl

S.No	Absorbance of Dox	Absorbance of Pyr	Doxylamine succinate (mcg/ml)	Pyridoxine HCl (mcg/ml)
1	0.7234	0.6566	20.970	25.799
2	0.7517	0.6853	26.9	26.85
3	0.7426	0.6553	18.5	26.6
4	0.7275	06553	17.75	26.21
5	0.7277	0.6548	19.95	26.43
6	0.7421	0.6546	19.62	25.6
AVG	0.7358	0.6603	20.6	26.2
SD	0.01	0.012	3.2	0.48
%RSD	0.013	0.018	0.15	0.018

Accuracy: The accuracy of the method was determined by measuring the drug recovery percentage by adding different amounts (80%, 100%, 120%) of bulk sample of doxylamine succinate and pyridoxine hydrochloride to reanalyzed formulation of concentration 25mcg/ml. from that percentage recovery values were calculated. The results were shown in table 4. The results obtained indicated that recovery percentage was between 98 – 102 % which indicated that the method was accurate for estimation of doxylamine succinate and pyridoxine hydrochloride in formulation the results were found within the acceptance criteria with acceptable no. %RSD of within 2%.

Table 7: Accuracy discussion data of Dorzolamide hydrochloride

Solution	Formln. (mcg/ ml)	Concentration (mcg/ml)		Amount found (mcg/ml)		%Recovery	
		Dox	Pyr	Dox	Pyr	Dox	Pyr
S_1	25	20.1	20.5	21.5	21.4	98.7	99.0
	25	19.9	20.30	20.3	20.8	99.5	98.5
	25	20.2	20.03	21.4	21.2	98.5	99.0
Mean % recovery						98.8	98.8
S.D						0.57	0.29
S_2	25	25.1	25.2	26.4	26.8	99.0	97.5
	25	25.3	25.22	25.8	26.9	99.3	98.4
	25	25.6	25.6	26.3	25.4	98.2	99.2
Mean % recovery						98.8	98.3
S.D						0.57	0.85
S_3	25	30.6	31.2	31.0	32.0	99.6	99.8
	25	30.2	31.2	30.5	31.9	98.8	98.7
	25	31.99	31.02	30.8	31.7	97.6	98.1
Mean % recovery						98.6	98.5
S.D						1.00	0.86

CONCLUSION

The proposed method was found to be simple, precise, and accurate and rapid for simultaneous estimation of doxylamine succinate and pyridoxine hydrochloride using water: methanol (70:30) as solvent. The linearity range was found to be 5-25 µg/ml for both the drugs. The proposed method was validated for parameters like specificity, accuracy, ruggedness was performed and ascertained values were found to be within limits. The method has significant advantages, in terms of shorter analysis time, selectivity, and accuracy then previously reported. The extraction method gave consistent and reproducible recovery for analyte from formulated preparation, with no interferences. The validation study

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indicates that method can be considered suitable. for carrying out quality control and routine determination of doxylamine succinate and pyridoxine hydrochloride under the study in bulk and pharmaceutical dosage form.

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