Rajani sekhar V. et al. / Vol 4 / Issue 2 / 2014 / 139-143.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

A VALIDATED SIMULTANEOUS ESTIMATION OF DOXYLAMINE SUCCINATE AND PYRIDOXINE HYDROCHLORIDE BY UV – SPECTROPHOTOMETRIC METHOD IN BULK AND FORMULATION

V. Rajani sekhar*, I.Sowkar Baig, R. Amulya, P. Nagarjuna

Department of Pharmaceutical Analysis and Quality Assurance, Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh, India- 517127.

ABSTRACT

A simple and rapid UV – Spectrophotometric method was developed and validated for the simultaneous estimation of Doxylamine succinate and Pyridoxine HCl in combined dosage forms. The said combination is used in treating morning sickness associated with pregnancy. For the simultaneous estimation of both drugs, 260 nm and 285 nm were selected as wavelength of analysis. The linearity range was taken 5-50µg/ml for Doxylamine and 2-18µg/ml for Pyridoxine which showed correlation coefficient of 0.9995 and 0.9998 respectively. The method was validated for accuracy and precision and % RSD was found to be within the acceptable limits. The percentage recoveries for both drugs were in the range of 95.5% – 98.33%. The LOD and LOQ of both drugs indicate that the method is sensitive. The assay of the method was in good agreement with the standards and enables the method to be adapted for routine analysis of the both drugs in combined dosage forms.

Keywords: Doxylamine succinate, Pyridoxine Hydrochloride, Simultaneous equations, Morning Sickness.

INTRODUCTION

Morning sickness is a common discomfort seen in most pregnant woman [1]. There was a need to treat this discomfort through a safe and effective medication so as to avoid birth defects. A combination of Doxylamine succinate, an anti – histamine in combination with Pyridoxine hydrochloride, Vitamin B6 was found to be safe for treating morning sickness during pregnancy as recommended by the guidelines of American college of Obstetricians and Gynecologists [2]. The chemical name for doxylamine succinate is ethanamine, N,N -dimethyl-2-[1phenyl-1-(2-pyridinyl)ethoxy]-, butanedioate and that of pyridoxine is 3,4-pyridinedimethanol, 5-hydroxy-6methyl-, hydrochloride [3]. The chemical structures of Doxylamine and Pyridoxine are shown in Figure 1.

Doxylamine is an antihistamine derived from monoethanolamine possessing antimuscarinic action and pronounced sedative effects [4]. Pyridoxine is a precursor of pyridoxal, which functions in the metabolism of carbohydrates, proteins and fats. It is essential in Hb formation and GABA synthesis within the CNS. It also aids in the release of glycogen stored in the liver and muscles. Till date, very few analytical methods are reported for the simultaneous estimation of Doxylamine and Pyridoxine Hydrochloride in dosage forms. The methods so far reported utilize derivative spectrophotometry [5], [6], HPLC [7], ratio derivative spectrophotometry [8], HPTLC [9] and charge transfer complexation [10] for the estimation of both the drugs. Analytical methods that involve simultaneous estimation of doxylamine and pyridoxine in combination with other drugs [11], [12] were also reported. A method using simultaneous equations was also reported [13]. The present method aims at developing a simple and rapid UV - spectrophotometric method using simultaneous equations for the estimation of both drugs in dosage forms.

Corresponding Author:- V. Rajani sekhar Email:- rajani6589@gmail.com

MATERIALS AND METHODS

Doxylamine succinate (99.94%) and Pyridoxine Hydrochloride (99.29%) were obtained Akums Drugs and Pharmaceuticals Limited, as gift samples. Methanol of AR grade was used as solvent. UV – Visible Double Beam Spectrophotometer – 2060, Analytical Technologies was used for measuring the absorbance.

Selection of Solvent

Solvents like methanol, acetontirile were selected based on solubility studies for both the drugs. Similar concentrations of both drugs were prepared in both solvents and scanned over the wavelength range of 200 – 400 nm. It was observed that, solutions in methanol showed suitable conditions for the simultaneous estimation of both drugs - Doxylamine having the maximum absorbance at 260 nm and Pyridoxine having the maximum absorbance at 285 nm, which were selected as the wavelength of analysis (Fig 2 and Fig 3). Methanol was selected as the solvent for further study.

Preparation of Standard Solutions

The standard stock solutions of Doxylamine and Pyridoxine were prepared by dissolving 10 mg of pure drug in 10 ml of methanol so as to give a concentration of 1 mg/ml. The working standards were prepared from the stick solutions by appropriate dilutions to give a concentration range of 5 – 50 µg/ml for Doxylamine and 2 – 20 µg/ml for Pyridoxine.

Preparation of Calibration Curve

The absorbance of the working standards was measured at 260 nm for Doxylamine and 285 nm for Pyridoxine and was plotted against concentration to get a calibration curve.

Simultaneous Equations method

The two wavelengths selected 260 nm and 285 nm were the wavelengths of maximum absorption of Doxylamine succinate and Pyridoxine HCl. The absorbance and absorptivities of both drugs were measured at the said wavelengths and the concentrations of the drugs were calculated using the simultaneous equation [14] as follows

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

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

C_x = concentration of Doxylamine Succinate

A_1 =absorbance of samples at 260 nm

C_y = concentration of Pyridoxine HCl

A_2= absorbance of samples at 285 nm.

ax_1 is the absorptivity of Doxylamine at 260 nm. ay_1 is the absorptivity of Doxylamine at 285 nm

ax_2 is the absorptivity of Pyridoxine at 260 nm. ay_2 is the absorptivity of Pyrdoxine at 285 nm

Method Validation

The present method was validated according to ICH Q2 Guidelines [15].

Linearity

The calibration curves were constructed with concentrations ranging from 5-50µg/ml for Doxylamine and 2-18µg/ml for Pyridoxine. The absorbance of the drug was considered for plotting the graph. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

The precision of the method can be determined through repeatability and Intermediate precision. The repeatability of the method was determined by taking six determinations of 100 % concentration (n = 6). The intermediate precision was determined by measuring the absorbance of the 100% concentration sample for two consecutive days.

Accuracy

The accuracy of the method was determined through percentage recovery studies. The pre analyzed sample was spiked with three different levels of reference standard solution and the absorbance was measured in triplicate. The percentage recovery was calculated individually for both drugs.

Limit of Detection and Quantitation

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated from standard deviation of response and slope.

Assay of Tablet Dosage Form

The present work was applied to estimate the drugs in tablet dosage forms. The test samples were prepared by weighing tablet powder equivalent to 10 mg of Doxylamine and Pyridoxine extracted with methanol and made the volume up to 10 ml with methanol. The working standard was prepared by appropriate dilution so as to get a concentration in the middle of linearity range. The absorbance was measured at 260 nm and 285 nm in triplicate. The results are shown in Table 6**.**

RESULTS AND DISCUSSION

Selection of Wavelength

The wavelengths of maximum absorbance for individual drugs were selected as the wavelength of analysis. The UV – Spectrum of both drugs is shown in Figure 2 and Figure 3.

Linearity

Standard solutions of Doxylamine and Pyridoxine were prepared and the absorbance was measured at 260 nm and 285 nm respectively. The linear regression parameters are summarized in Table 1.

Precision

The repeatability of the method is expressed as % RSD and is found to be 0.784 and 0.487 for Doxylamine and Pyridoxine respectively. The intermediate precision is expressed as % RSD and the inter day study is found to be 1.019 and 0.482 for Doxylamine and Pyridoxine respectively. The results are summarized in Table 2.

Accuracy

The accuracy of the method was determined by calculating percentage recovery of three levels in triplicate. The results are shown in Table 3 and Table 4.

LOD and LOQ

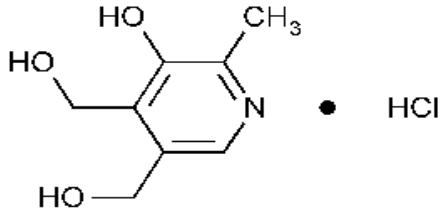
The limit of detection and limit of quantitation were calculated from standard deviation of response and slope and were found to be 0.1815 µg/ ml and 0.551 µg/ml respectively for Doxylamine and 0.1941 µg/ml and 0.558 µg/ml respectively for Pyridoxine.

Assay of Tablet Dosage Form

The developed method was applied to tablet dosage form and the results obtained were in good agreement with the label claim for both drugs. The results are shown in Table 5.

Figure 1. Chemical structures of Doxylamine and Pyridoxine

Pyridoxine Hydrochloride



Doxylamine succinate

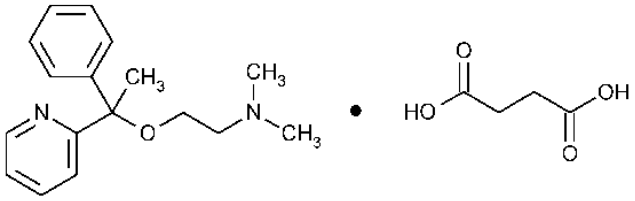


Figure 2. UV Spectrum of Doxylamine showing λ_{max} at 260 nm

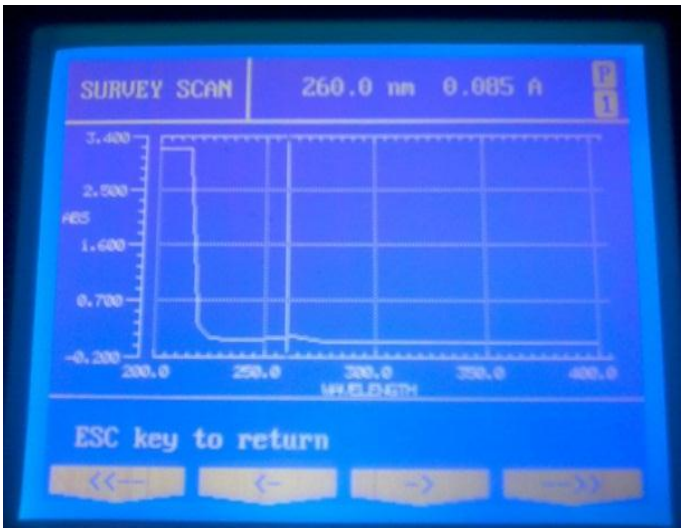


Figure 3. UV Spectrum of Pyridoxine showing λ_{max} at 285 nm

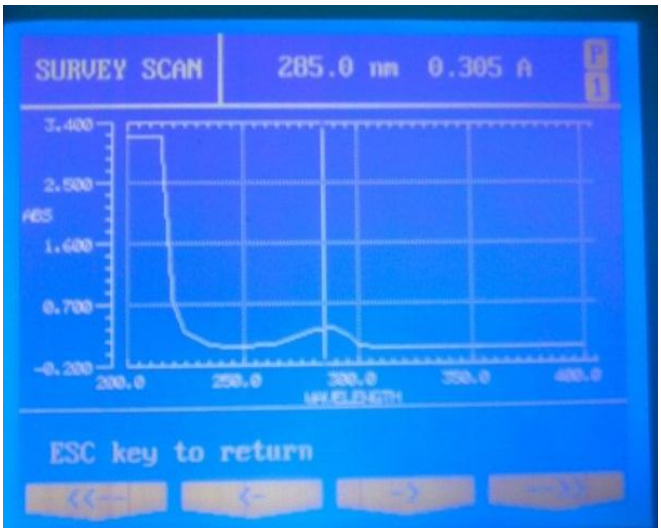


Table 1. Linear Regression data of Doxylamine and Pyridoxine

Parameters	Doxylamine	Pyridoxine
Number of Samples	10	10
Wavelength (nm)	260	285
Linearity range (µg/ml)	5 - 50	2 - 20
Correlation Coefficient	0.9995	0.9998
Regression line equation	y = 0.023x + 0.015	y = 0.030x – 0.001

Table 2. Repeatability studies of Doxylamine and Pyridoxine

Set No.	Absorbance of Doxylamine at 260 nm	Absorbance of Pyridoxine at 285 nm
1	0.115	0.300
2	0.113	0.301
3	0.114	0.304

4	0.113	0.303
5	0.114	0.302
6	0.115	0.303
Mean	0.114	0.3021
Standard Deviation	0.000894	0.00147
% RSD	0.784	0.487

Table 3. Accuracy of Doxylamine

Recovery level	Amount added (µg/ml)	Amount found (µg/ml) Avg ± SD	% Recovery Avg ± SD	% RSD
80 %	8	7.7 ± 0.02624	96.25 ± 0.282	0.293
100%	10	9.73 ± 0.119	97.3 ± 1.131	1.162
120%	12	11.80 ± 0.850	98.33 ± 0.452	0.4596

Table 4. Accuracy of Pyridoxine HCl

Recovery level	Amount added (µg/ml)	Amount found (µg/ml) Avg ± SD	% Recovery Avg ± SD	% RSD
80%	8	7.64 ± 0.110	95.5 ± 0.848	0.8879
100%	10	9.69 ± 0.166	96.9 ± 1.414	1.459
120%	12	11.74 ± 0.199	97.83 ± 0.965	0.9864

Table 5. Assay of Tablet Formulation

Sample	Label Claim	Amount Found	% Assay (Avg ± SD)	% RSD
Doximine tablets	Doxylamine 10mg	10.092 ± 0.0007	100. 92 ± 0.701	0.6946
	Pyridoxine 10mg	9.161 ± 0.0855	91.61 ± 0.855	0.9330

CONCLUSION

A simple, sensitive, economic and rapid technique for the estimation of Doxylamine Succinate and Pyridoxine Hydrochloride in dosage forms was developed. This method proved to be simple when compared to the other methods reported. The lower values Limit of Detection and Limit of Quantitation of when compared to the other spectrophotometric methods developed, proves the sensitivity of technique. The good agreement of validation parameters and the assay with standards enables the method to be adapted for routine analysis of both the drugs in dosage forms.

REFERENCES

1. ACOG Issues Guidance on Treatment of Morning Sickness during Pregnancy, ACOG NEWS RELEASE, 2004.
2. Arayne MS, Sultana et al. Spectrophotophotometric methods for the Simultaneous analysis of Meclezine Hydrochloride and Pyridoxine Hydrochloride in bulk drug and pharmaceutical formulation. *Pakistan Journal of Pharma Sciences*, 20(2), 2007, 149 -156.
3. Argekar A, Sawant J. Simultaneous determination of pyridoxine hydrochloride and doxylamine succinate from tablets by ion pair reversed-phase high-performance liquid chromatography (RP-HPLC). *Drug Development and Industrial Pharmacy*, 25(8), 1999, 945-950.
4. Argekar AP and Sawant JG Simultaneous Determination Of Pyridoxine Hydrochloride and Doxylamine Succinate in Tablets by HPTLC. *Journal of Liquid Chromatography & Related Technologies*, 22 (13), 1999, 2051 – 2060.
5. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry: part II.4th ed. CBS Publications and Distributors. 1997, 284 – 286.
6. Comparative pharmacokinetics of single doses of doxylamine succinate following intranasal, oral and intravenous administration in rats, Available from: URL: http://www3.interscience.wiley.com/journal/98016819/abstract
7. Gadsby R, Barnie-Adshead AM, Jagger CA. Prospective study of nausea and vomiting during pregnancy. *Br J Gen Pract*, 43, 1993, 245-248.
8. International Conference on Harmonisation, ICH Harmonised Tripartite Guideline- Validation of Analytical Procedures: Methodology. Fed Reg, 62, 1997, 27463.
9. O'Neil MJ. The Merck Index, 14th ed. Merck & Co. USA, 2006, 583 & 1373.
10. Nataraj KS, Suvarna Y et al. Development and Validation of method for Simultaneous estimation of Pyridoxine Hydrochloride and Doxylamine Succinate in tablet dosage forms by First order Derivative Spectroscopy. *International Journal of Pharmacy*, 5(1), 2012, 388 – 390.

11. Patak A, Rajput SJ et al. Simultaneous Derivative Spectrophotometric Analysis of Doxylamine Succinate, Pyridoxine Hydrochloride and Folic acid in Combined Dosage Forms. *Indian Journal of Pharmaceutical Sciences*, 70 (4), 2008, 513-517.
12. Pathak A, Rajput S. Simultaneous determination of ternary mixture of Pyridoxine Hydrochloride, Doxylamine Succinate and folic acid by a ratio spectra zero crossing, double divisor-ratio spectra derivative and column HPLC methods. *J AOAC Int* 2008; 91(5): 1059-1069.
13. Pratap Y, Pawa et al. Simultaneous UV Spectrophotometric Method for Estimation of Isoniazid and Pyridoxine in Tablet Dosage Form. *Scholar Research Library, Derpharma chemica*, 4 (2), 2012, 749-754.
14. Raza A, Ansari T, Niazi S, Rehman A. Spectrophotometric Determination of pyridoxine Hydrochloride (Vitamin B6) in Bulk and Tablets by Charge- Transfer Complexation with Chloranil. *J Chem Soc Pakistan*, 29(1), 2007, 33-36.
15. Smita C .Nayak, Preeti .V. Kulkarni, Vaidhun Bhaskar, Vinuth Chavhan, Development and validation of UV – Spectrophotometric method for simultaneous estimation of Doxylamine succinate and Pyridoxine hydrochloride in bulk and tablet dosage form. *International journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 2013, 390 – 393.