Advanced Journal of Chemistry-Section A, 2019, 2(3), 245-255
Research Article
http://ajchem-a.com

Validated UV-Spectrophotometric Method for the Simultaneous Estimation of Pyridoxine Hydrochloride and Doxylamine Succinate in Bulk and in Pharmaceutical Dosage form

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ARTICLE INFO

Received: 16 February 2019
Revised: 19 February 2019
Accepted: 4 March 2019
Available online: 16 March 2019

DOI: 10.29088/SAMI/AJCA.2019.2.245255

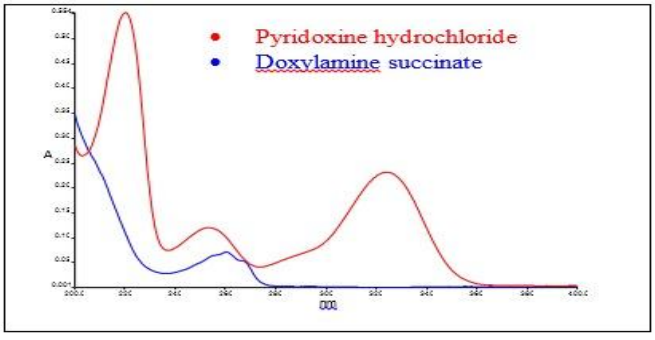
KEYWORDS

Pyridoxine Hydrochloride
Doxylamine Succinate
Absorbance correction method
Validation

ABSTRACT

A new, simple, accurate, and sensitive UV - Spectrophotometric absorbance correction method has been developed for simultaneous determination of Pyridoxine Hydrochloride and Doxylamine Succinate in bulk and in combined tablet dosage form using distilled water as a solvent. The wavelengths selected for the analysis were 260 nm and 324 nm. Both Pyridoxine hydrochloride and Doxylamine Succinate were linear over the concentration range of 5 - 40 µg/ mL and 10 - 60 µg/ mL of Doxylamine Succinate and Pyridoxine hydrochloride, respectively. The percentage recovery was found to be in the range of 99.15 - 100.71% for Pyridoxine Hydrochloride and 99.30 - 101.99% for Doxylamine Succinate. The %RSD for recovery studies was found to be 0.5484 and 0.9071 for Pyridoxine hydrochloride and Doxylamine Succinate, respectively. The low %RSD of recovery studies indicated that there is no interference due to excipients used in formulation. The amount of Pyridoxine hydrochloride and Doxylamine succinate was found to be 100.92% ± 0.6961 and 101.05% ± 0.7965. Optical characteristics like slope, intercept, molar absorptivity, correlation coefficient, LOD and LOQ were calculated. The developed method was validated statistically by recovery studies as per ICH guidelines. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, rapid and accurate and can be successfully applied for routine quality control analysis of simultaneous determination of Pyridoxine Hydrochloride and Doxylamine Succinate in bulk and in combined tablet dosage form.

GRAPHICAL ABSTRACT



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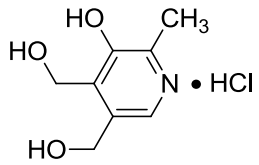
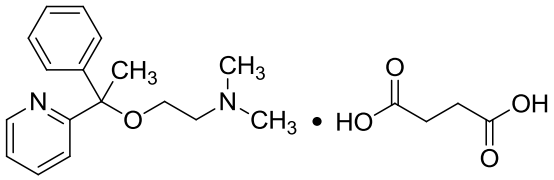
Introduction

Pyridoxine Hydrochloride (PYRI), chemically it is 5-hydroxyl-6methyl pyridine 3, 4 dimethanol [1] (Figure 1). Pyridoxine is converted in erythrocytes to pyridoxal phosphate and to a lesser extent pyridoxamine phosphate which act as coenzymes for various metabolic functions affecting protein, carbohydrate, and lipid utilization [2]. It is official in IP [3], BP [4] and USP [5].

Doxylamine Succinate (DOXY), chemically it is dimethyl ({2-[1-phenyl-1-(pyridin-2-yl) ethoxy] ethyl}) amine (Figure 2). Doxylamine Succinate is an antihistamine, used to relieve symptoms of allergy, hay fever, and the common cold. This effect helps to relieve allergy, cold symptoms such as watery eyes, runny nose, and sneezing [6, 7]. Also used in the combination with Vitamin B6 (pyridoxine) to prevent morning sickness in pregnant women [8]. The combined dosage forms of DOXY and PYRI are available in the market for the treatment of vomiting during pregnancy. It is official in BP [9] and USP [10].

Literature survey revealed that numbers of RP-HPLC methods are available for DOXY in combination with other drugs [11-14] and also PYRI in combination with other drugs [15-17]. RP-HPLC method was also reported for the simultaneous estimation of DOXY and PYRI in combination [18-20]. UV Spectrophotometric method like simultaneous equation method [21-23] and First derivative Spectrophotometry [24] were reported.

Figure 1. Structure of pyridoxine hydrochloride	
Figure 2. Structure of doxylamine succinate	

The present study aims to develop a simple, sensitive, accurate, precise, reproducible and rapid UV Spectrophotometric method for the estimation of DOXY and PYRI in bulk and in combined tablet dosage form by using absorbance correction method.

Materials and methods

Apparatus

A Perkin Elmer model Lambda 25 double beam UV-Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell were used to measure absorbance of all the solutions. A Shimadzu AUX 220 electronic balance was used in the study.

Reagents and Materials

PYRI and DOXY bulk powder was kindly gifted by Sai Mirra Inno pharm Pvt Ltd, Chennai, India. The commercial fixed dose combination of Doxinate tablet containing 10 mg of Pyridoxine Hydrochloride and 10 mg of Doxylamine Succinate was procured from the local market. Calibrated glass wares were employed throughout the work.

Experimental condition

According to the solubility characteristics, the common solvent for the two drugs was found to be distilled water. Hence the stock solution was prepared using distilled water.

Preparation of standard stock solution

100 mg of PYRI and 100 mg of DOXY were accurately weighed and transferred in to 100 ml volumetric flasks separately. Dissolved in distilled water and made up to the volume to 100 ml with the same. These solutions were observed to contain 1 mg/ mL of both PYRI and DOXY, respectively.

Study of spectral and linearity characteristics

The standard stock solutions of PYRI and DOXY were further diluted with distilled water to get the concentration of 10 µg/ mL of each and the solutions were scanned between the range 200 - 400 nm in 1 cm cell against distilled water as blank. The overlain spectra were recorded. From the overlain spectrum of PYRI and DOXY, it was observed that DOXY have zero absorbance at 324 nm, whereas PYRI has substantial absorbance. Hence, PYRI was estimated directly at 324 nm without interference of DOXY. At 260 nm, these two drugs were showed the absorbance. To estimate the amount of DOXY, the absorbance of PYRI were corrected for interference at 260 nm by using their absorptivity values.

Preparation of calibration graph

The standard stock solution of PYRI (0.5 - 4 mL) and DOXY (1 - 6 mL) was transferred into a series of 100 ml volumetric flask and made up to the volume with distilled water. The absorbance of different concentration

solutions were measured at 260 nm and 324 nm. The calibration curve was constructed by plotting concentration Vs absorbance. PYRI was linear with the concentration range of 5–40 µg/mL at 260 and 324 nm and DOXY was linear with the concentration range of 10–60 µg/ mL at 260 nm.

Analysis of synthetic mixture of DOXY and PYRI

Different mixtures of the two drugs were prepared by transferring different volumes of PYRI and DOXY from standard stock solutions into 100 mL volumetric flasks and diluting to volume with distilled water. The concentrations of all the two drugs were determined by measuring the absorbance of the prepared mixtures at 324 nm and 260 nm. From these absorbance values, the concentrations of PYRI and DOXY were determined by using absorbance correction method.

Analysis of tablet formulation

Ten tablets were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 100 mg of Pyridoxine Hydrochloride was transferred in to 100 mL volumetric flask and added a minimum quantity of distilled water to dissolve the substance and made up to the volume with the same. The solution was sonicated for 15 minutes, centrifuged for another 15 minutes at 100 rpm and filtered through the Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 2 mL into 100 ml with distilled water to obtain 20 µg/mL solution of PYRI which is also contains 20 µg/mL of DOXY theoretically. The absorbance of sample solution was measured at all selected wavelengths. The content of PYRI and DOXY in sample solution was calculated. This procedure was repeated for six times.

Recovery studies

Preparation of DOXY and PYRI raw material stock solution

400 mg of PYRI and DOXY was accurately weighed and transferred into 10 mL volumetric flask individually and sufficient distilled water is added to dissolve the substance and made up to the mark with the same. Both the stock solution contains 40 mg/mL concentration.

Procedure

The recovery experiment was done by adding known concentrations of raw material stock solution of PYRI and DOXY to the pre-analyzed formulation. The tablet powder equivalent to 100 mg of PYRI was weighed accurately and added 2 mL, 2.5 mL and 3 mL of above raw material stock solutions into a series of 100 mL volumetric flask and dissolved with distilled water and

sonicated for 15 minutes. The solution was made up to 100 mL with distilled water and centrifuged for 15 minutes at 2000 rpm. The supernatant liquid was filtered through a Whatmann filter paper No.41. 2 ml of the clear solution was transferred into 100 mL volumetric flask and made up to 100 mL with distilled water. The absorbance of three replicates was measured at their selected wavelengths. The amount of drug recovered from formulation was calculated. The procedure was repeated for three times for each concentration.

Validation

The method was validated as per ICH guidelines [25]. The methods were validated with respects to linearity, LOD (Limit of detection), LOQ (Limit of quantitation), precision, accuracy and ruggedness. The validation parameters are explained as given below.

Linearity

Linearity was checked by diluting standard stock solution at different concentrations. PYRI was linear with the concentration range of 5-40 µg/mL at 260 and 324 nm. DOXY was linear with the concentration range of 10-60 µg/mL at 260 nm. Calibration curves (n=6) were plotted between concentration and absorbance of drugs. Optical parameters such as slope, intercept, molar absorptivity, correlation coefficient, LOD and LOQ were calculated.

Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations; LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is standard deviation of y intercept of calibration curve (n=6) and S is slope of regression equation

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis *i.e.* the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated.

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations viz, 80%, 100% and 120%. From the total amount of drug found, the

percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In present study, determination of DOXY and PYRI were carried out by using different analysts.

Results and discussion

A simple, precise, accurate and rapid UV Spectrophotometric method was developed for the estimation of DOXY and PYRI in bulk and in formulation by using absorbance correction method. From the solubility data, distilled water was used as a common solvent for the analysis of these drugs. The spectrum of PYRI and DOXY was reported in the wavelength range of 200-400 nm and overlain. From the overlain spectrum, the wavelengths selected for the analysis were 324 nm and 260 nm (Figure 3).

Based upon the spectral characteristics of both the drugs, absorbance correction method was selected for analysis. At 260 nm both PYRI and DOXY were have marked absorbance. At 324 nm PYRI had absorbance but DOXY is zero. Hence at 324 nm PYRI could be analyzed without the interference of DOXY. After determining the amount of PYRI at 260 nm the absorbance of PYRI was corrected to determine the amount of DOXY. Hence these two wavelengths were selected for the simultaneous estimation of PYRI and DOXY in combination without prior separation.

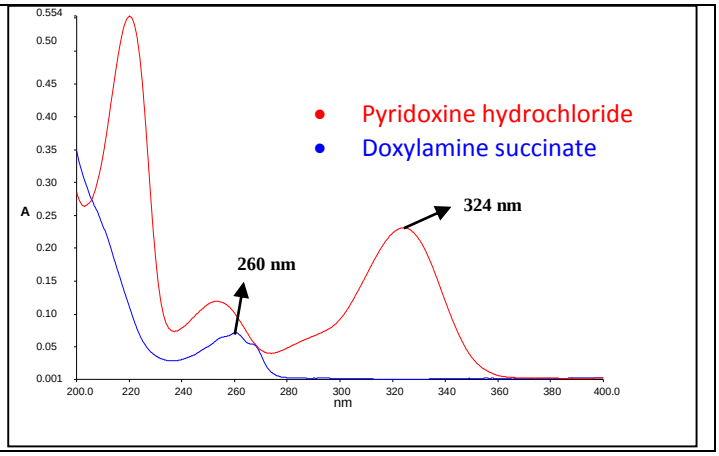


Figure 3. Uv overlain spectra of pyridoxine hydrochloride and doxylamine succinate (10 µg/ mL)

The stability of PYRI and DOXY was studied by measuring the absorbance at different time intervals. It was observed that PYRI was stable up to 6 hours in distilled water and DOXY was stable up to 1 hour 30 minutes in distilled water.

Validation Data of the Proposed Methods

Beer's law obeyed in the concentration range of 5 - 40 µg/mL at 324 nm and 10-60 µg/mL at 260 nm for PYRI and DOXY, respectively. The correlation coefficient values were found to be above 0.999, which shows that absorbance of all the drugs was linear with concentration. The optical characteristics such as Beer's law limits, correlation Coefficient, slope, intercept, Sandell's sensitivity and molar absorptivity were calculated and are summarized in (Table 1).

The LOD and LOQ were found to be 0.1539 and 0.4664for PYRI and 0.0318 and 0.0964 for DOXY, respectively. The low values indicated that the sensitivity of the method.

To study the mutual interference, if any, in the simultaneous estimation of synthetic mixture containing various proportions of PYRI and DOXY were prepared and the contents were estimated by proposed method. The % Recovery varied from 100.05 - 101.85% for PYRI and 99.99 - 102.11% for DOXY indicating that there is no mutual interference between these two drugs. The result of analysis of synthetic mixture is shown in (Table 2).

The percentage label claim present in tablet formulation was found to be 101.32±1.2453 for PYRI and 100.17% ± 0.8898 for DOXY, respectively. Precision of the method was confirmed by the repeated analysis of formulation for six times. The % RSD values were found to be 1.2291 for PYRI and 0.8882 for DOXY, respectively.

Table 1. Optical characteristics of pyri and doxy at the selected wavelengths

Parameters	Pyridoxine Hydrochloride	Doxylamine Succinate
λmax (nm)	324 nm	260 nm
Beer's law limit (µg/ ml)	5 – 40	10 – 60
Sandell's sensitivity (µg/ cm^2/ 0.001 A.U)	0.0304	0.0980
Molar absorptivity (L mol^{-1} cm^{-1})	6890.0938	2792.8950
Correlation coefficient (r)	0.9999	0.9998
Regression equation (y = mx + c)	Y = 0.0329x + 0.0058	Y = 0.0101x + 0.0013
Slope (m)	0.0329	0.0101
Intercept (c)	0.0058	0.0013
LOD (µg/ ml)	0.1539	0.0318
LOQ (µg/ ml)	0.4664	0.0964
Standard error	0.0068	0.0044

*Mean of six observations

Table 2: Results of analysis of synthetic mixtures

Concentration of Pyridoxine Hydrochloride (µg/ ml)		% Recovery	Concentration of Doxylamine Succinate (µg/ ml)		% Recovery
Theoretical	Experimental		Theoretical	Experimental	
10	9.8728	100.05	40	40.3551	101.08
15	15.2786	101.85	35	35.3835	101.09
20	20.1337	100.62	30	30.5584	101.85
25	25.0522	100.20	25	25.1925	100.10
30	30.4058	101.35	20	20.2243	101.11
35	35.5613	101.60	15	15.3194	102.11
40	40.2989	100.74	10	9.9997	99.99

Table 3: Results of analysis of tablet formulation

Parameters	Pyridoxine Hydrochloride	Doxylamine Succinate
Label Claim (mg)	10 mg	10 mg
% Assay*	101.32	100.17
SD	1.2453	0.8898
% RSD	1.2291	0.8882

*Mean of six observations

The low % RSD values indicated that both drugs showed good agreement with the label claim ensures the precision of the method were shown in (Table 3).

Further, the precision of the method was confirmed by Intraday and Inter day analysis. The % RSD values for intraday and inter day analysis was found to be 0.2603 and 1.1609 for DOXY, 0.1891 and 0.19920 for PYRI, respectively. Hence the precision of the method was further confirmed (Table 4).

The developed method was validated for Ruggedness. The analysis of formulation was done by using different analysts. The % RSD values were found to be less than 2 indicating that the method was more rugged. The results of analysis of intermediate precision and ruggedness are shown in (Table 4).

In order to check the accuracy of the developed method, known quantities of standard drugs of PYRI and DOXY in three different concentrations were added to its pre analyzed sample and analyzed by the developed method. The percentage recovery was found to be in the range of 99.31-100.45% for PYRI and 99.55-101.49% for DOXY. The results of recovery studies are shown in (Table 5). The % RSD values for PYRI and DOXY were found to be 0.5484 and 0.9017, respectively. The low % RSD values confirmed that there is no interference due to the excipients used in formulation. This ensures the accuracy of the method.

Table 4: Intermediate precision and ruggedness of the method

Parameters	% Label claim estimated (Mean ± % RSD)	
	Pyridoxine Hydrochloride	Doxylamine Succinate
Intraday Precision (n=3)	101.78 ± 0.1891	100.95 ± 0.2603
Inter day Precision (n=3)	101.69 ± 1.9920	99.93 ± 1.1609
Different analysts (n=6)		
Analyst I	101.64 ± 0.2673	100.50 ± 0.3306
Analyst II	101.27 ± 0.1786	100.23 ± 0.5139

*Mean of six observations

Table 5. Recovery studies

Drug	Amount present (µg/ mL)	Amount added (µg/ mL)	Amount found* (µg/ mL)	Amount recovered (µg/ mL)	% Recovery*	SD	% RSD
Pyridoxine	20.2639	16.0759	36.4150	16.1510	100.45		
Hydrochloride	20.2639	20.1337	40.4139	20.1500	100.07	0.5481	0.5484
	20.2639	24.2558	44.3549	24.0910	99.31		
				Mean %	99.954		
Doxylamine	20.0350	16.2739	36.2365	16.2015	99.55		
Succinate	20.0350	20.2013	40.5393	20.5043	101.49	0.9073	0.9017
	20.0350	24.0297	44.2580	24.2230	100.79		
				Mean %	100.805		

*Mean of three observations

Conclusion

From validation, the developed method was found to be simple, rapid, economical, precise, accurate and rugged. Hence the proposed method could be effectively applied for the routine quality control analysis of PYRI and DOXY in bulk and in combined tablet dosage form.

Acknowledgement

The authors are very much thankful to Dr. G. Murugananthan, Principal, Swamy Vivekanandha college of Pharmacy, Tiruchengode, for his support and also thankful to the management for providing the necessary facilities to carry out the research work successfully.

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How to cite this manuscript: Sangeetha Premkumar, Anandkumar Karunakaran*, Vasanthi Murugesan, Jambulingam Munusamy, Ramesh Jayaprakash, Raja Murugesan, Validated UV-Spectrophotometric Method for the Simultaneous Estimation of Pyridoxine Hydrochloride and Doxylamine Succinate in Bulk and in Pharmaceutical Dosage form, *Advanced Journal of Chemistry-Section A (Adv. J. Chem. A)*, **2019**, *2*(3), 245-255.