

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

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ABSTRACT

Objective: To develop simple, rapid UV spectrophotometric method for simultaneous determination of Pyridoxine hydrochloride (PYR) and Doxylamine succinate (DOX) in bulk and tablet dosage form by simultaneous equation method.

Methods: Solubility studies were carried out to identify a suitable solvent for dissolving both PYR and DOX. Next, both drugs were dissolved individually as well as in combination in the solvent and their λ_{\max} values as well as absorption pattern over the wavelength 200 to 400 nm were studied. The data obtained was used to derive simultaneous equations for determining the concentrations of the two drugs. Different parameters of study were optimized and the method was validated as per ICH guidelines.

Results: PYR and DOX showed absorption maxima at 290 and 260 nm respectively. They also showed significant absorption at each other's maxima. Standard curves of both drugs obeyed Beer- Lambert's law in concentration range of 4-20 $\mu\text{g/ml}$. Simultaneous equations were developed and validated as per ICH guidelines for accuracy, linearity and precision. Marketed sample of tablets was analyzed by using the validated method and yielded accurate results.

Conclusion: The method was found to be simple, accurate, precise, reproducible, economic and suitable for routine quality control analysis as well as validation of in-process parameters.

Keywords: Nausea, vomiting, Pyridoxine hydrochloride, Doxylamine succinate, validation, ICH guidelines.

INTRODUCTION

Nausea and vomiting in pregnancy (NVP) affect 70–85% of pregnant women [1]. The degree of morning sickness symptoms can range from mild to severe (hyperemesis gravidarum) [2]. The guidelines given by The American College of Obstetricians and Gynecologists recommend administration of Vitamin B6 (Pyridoxine) or Vitamin B6 with

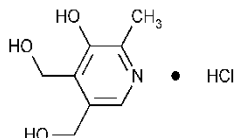


Fig. 1: It shows chemical structure of Pyridoxine hydrochloride.

Doxylamine for the prevention and treatment of NVP. This combination has been found to be safe and effective and is considered the first line of treatment [3]. Pyridoxine hydrochloride is chemically, 5-hydroxy-6-methyl-3,4-pyridinedimethanol hydrochloride (Fig.1) and Doxylamine succinate is N,N-dimethyl-2-[1-phenyl-1-(2-pyridinyl) ethoxy]-butanedioate (Fig.2) [4]. As per I.P., PYR is evaluated by perchloric acid titration method [5]. DOX is official in U.S.P. and is analyzed by perchloric acid titration [6]. A literature survey was carried out and revealed that many analytical methods like HPLC [7-9], UV Spectroscopy [10-11] and charge transfer complexation [12] methods have been reported for determination of PYR and DOX combination. No official method exists for simultaneous estimation of these drugs from the combination. Therefore objective of this study was to develop a simple simultaneous UV spectroscopic method for rapid and accurate estimation of individual drug content from the mixture.

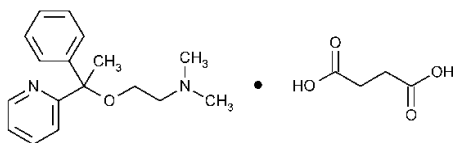


Fig.2: It shows chemical structure of Doxylamine succinate.

MATERIALS AND METHODS

Reagents

Pyridoxine hydrochloride I.P. and Doxylamine succinate U.S.P. were obtained as free gift samples from Maneesh Pharmaceuticals Pvt. Ltd., Mumbai. The pharmaceutical preparation i.e. the combination tablet is procured from local market. Remaining all the reagents and solvents were of Analytical grade and purchased from Merck Chemicals, India while double distilled water was used for whole experiment.

Instrumentation

UV-visible double beam spectrophotometer, Lab India, UV 3000+ with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used.

Preliminary solubility studies of drugs

Accurately about 100 mg of each drug was weighed and solubility was checked in water, methanol, ethanol, 0.1N HCl, 0.1 N NaOH. Both the drugs were soluble in 0.1N HCl.

Preparation of standard stock solution

Accurately about 25 mg of each drug was weighed and dissolved in 10 ml 0.1 N HCl and sonicated for 15 minutes. The volume was made up to 25 ml with 0.1 N HCl to obtain standard stock solution (1000 $\mu\text{g/ml}$). From this, 2.5 ml solution was withdrawn and diluted upto 25 ml with 0.1 N HCl (100 $\mu\text{g/ml}$) and used as working stock solution. This solution was serially diluted to get concentration in the range of 4-20 $\mu\text{g/ml}$.

Preparation of Calibration curve

From the standard stock solution appropriate dilutions were made to obtain concentration in range of 4, 8, 12, 16 and 20 $\mu\text{g/ml}$ for both drugs. The spectra were recorded, absorbances were measured at 290 nm and 260 nm for PYR and DOX respectively and calibration curves were plotted.

Sample preparation

Tablets containing PYR and DOX in combination were procured from local market and average weight of 20 tablets was determined.

Powder equivalent to 25 mg of each drug was weighed accurately and transferred to 25 ml volumetric flask. The drugs were extracted into 10 ml of 0.1N HCl by sonication, filtered through Whatmann filter paper and volume was made up to 25 ml with 0.1N HCl (1000 µg/ml). From this solution, 2.5 ml was withdrawn and diluted to 25 ml with 0.1N HCl (100 µg/ml). From this further dilution was made for estimation of drug content.

Simultaneous Equation Method

Two wavelengths selected for the method namely, 290 nm and 260 nm are absorption maximas of DOX and PYR respectively in 0.1N HCl (Fig. 3). The stock solutions of both the drugs were further diluted separately with 0.1 N HCl to get a series of standard solutions of 4-20 of µg/ml concentrations of both drugs. The absorbances as well

as absorptivity values of all solutions were measured at both the wavelengths 260 nm and 290 nm respectively and substituted in the following equation.

$$C_x = (A_2a_{y1} - A_1a_{y2}) / (ax_2a_{y1} - ax_1a_{y2})$$

And

$$C_y = (A_1ax_2 - A_2ax_1) / (ax_2a_{y1} - ax_1a_{y2})$$

C_x = concentration of PYR A₁=absorbance of samples at 290 nm

C_y = concentration of DOX A₂= absorbance of samples at 260 nm.

ax₁ is the absorptivity of PYR at 290 nm. ay₁ is the absorptivity of PYR at 290 nm.

Table 1: It shows Optical characteristics of both drugs.

Parameters	PYR	DOX
λ _{max} (nm)	290 nm	260 nm
Beer's range (µg/ml)	4-20 µg/ml	4-20 µg/ml
Correlation coefficient (r ²)	0.9999	1
Regression equation	Y = 0.0265x + 0.0129	Y = 0.043x + 0.0029
Intercept (a)	0.0129	0.0029
Slope (b)	0.0265	0.043

ax₂ is the absorptivity of DOX at 260 nm. ay₂ is the absorptivity of DOX at 260 nm.

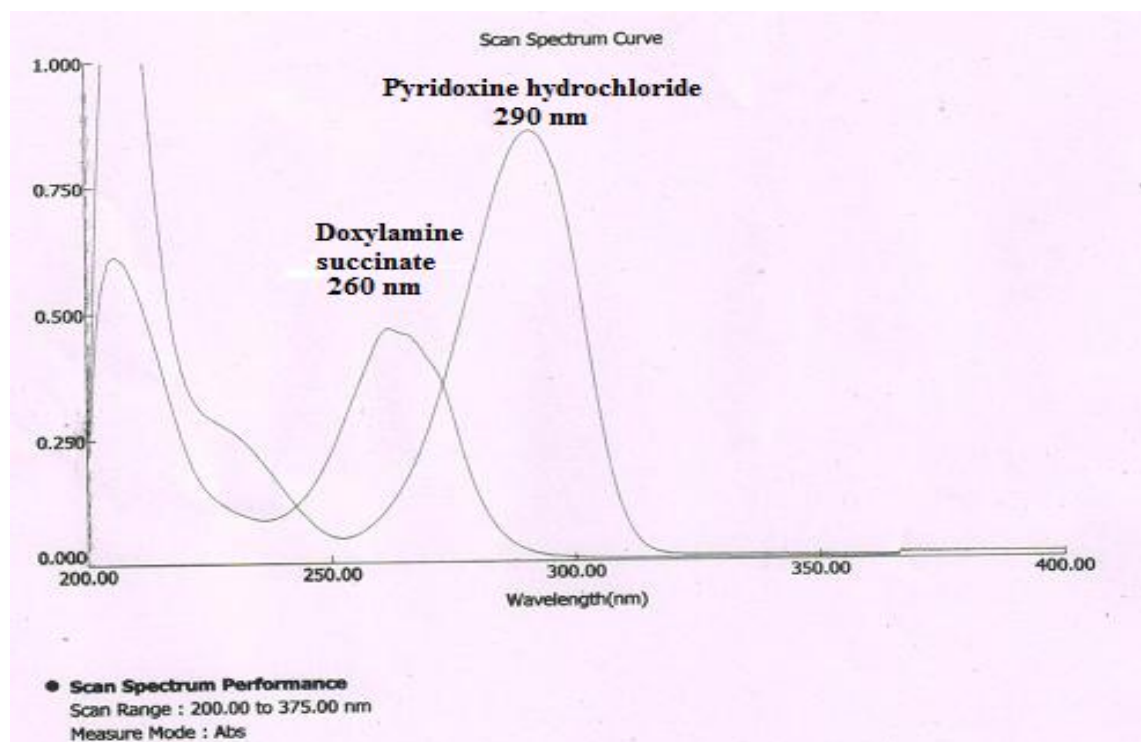


Fig. 3: It shows overlain spectra of Pyridoxine hydrochloride and Doxylamine succinate in 0.1N HCl. X axis depicts wavelength in nm and Y axis depicts absorbance.

Method Validation

Validation of method of analysis of PYR and DOX in bulk and pharmaceutical formulations (marketed and developed) was carried out as per ICH guidelines Q₂ (R₁) [13].

RESULTS

Simultaneous equations were developed in order to determine concentrations of PYR and DOX. Upon substitution of absorptivity values, the equations were further simplified to

Concentration of pyridoxine hydrochloride in g/100 mL.

$$= \frac{A_2 \cdot 208 - A_1 \cdot 11}{87915} \quad \text{(Equation 1)}$$

Concentration of doxylamine succinate in g/100 mL.

$$= \frac{A_1 \cdot 425 - A_2 \cdot 44}{87915} \quad \text{(Equation 2)}$$

Where A₁ is the absorbance of the sample at 260 nm

A₂ is the absorbance of the sample at 290 nm

Method Validation

Linearity and Range

Calibration curve constructed was linear over the selected range of 4-20 µg/ml for both drugs at λ_{max} of 290 nm and 260 nm respectively. Each concentration was repeated three times. The

assays were performed according to experimental conditions and the linearity of the calibration graphs were validated by the high value of the correlation coefficient and the intercept value (Table 1).

Accuracy

Accuracy of the developed method was confirmed by doing recovery study of both drugs as per ICH guidelines at three different concentration levels- 80%, 100%, 120% by replicate analysis (n=3). This study was performed by addition of known amounts of both pure drugs to a known concentration of the commercial tablets. The amount of standard recovered was calculated in the terms of mean

recovery with the percent Relative Standard Deviation (% RSD) (Table 2).

Precision

Precision was determined by studying the repeatability which indicates the precision under the same operating conditions over a short interval time. The experiments were repeated six times for precision. The developed method was found to be precise for intraday and inter day precision on the basis of % RSD values for both drugs (Table 3 and 4).

Table 2: It shows results of Recovery Studies.

Sr. No	Amount Added %	PYR Conc. % recovery \pm S.D.	% COV	DOX Conc. % recovery \pm S.D.	% COV
1.	80	100.181 \pm 1.126	0.521	100.36 \pm 0.943	0.445
2.	100	99.416 \pm 0.57	0.418	99.685 \pm 1.29	0.628
3.	120	100.04 \pm 0.75	0.323	100.177 \pm 1.105	0.467

Results are mean of three readings and expressed as mean \pm standard deviation,

Table 3: It shows results of Intraday Precision Studies.

Sr. No.	Conc. (mcg/mL)	PYR Conc. (%)	% COV	DOX Conc. (%)	% COV
1.	20	100.14 \pm 1.403	0.543	102.023 \pm 0.796	1.011

Results are mean of six readings and expressed as mean \pm standard deviation,

Table 4: It shows results of Interday Precision Studies.

Sr. No.	Conc. (mg/ml)	PYR Conc. (%)	% COV	DOX Conc. (%)	% COV
1.	20	99.63 \pm 1.8	0.809	99.905 \pm 0.998	0.392

Results are mean of six readings and expressed as mean \pm standard deviation.

Table 5: It shows Results of LOD and LOQ.

Active Ingredient	LOD (μ g/ml)	LOQ (μ g/ml)
PYR	0.580	1.750
DOX	0.323	0.979

Table 6: It shows Results of Tablet Assay.

Active Ingredients	Label Claim (mg/tab)	Amount of Drug Estimated (mg/tab)	% Assay
PYR	10 mg	9.85	98.5
DOX	10 mg	9.91	99.1

Results are mean of six readings.

DISCUSSION

The present paper describes estimation of PYR and DOX in bulk and tablet dosage form by Simultaneous equation method. Solubility studies indicated that both drugs shows better solubility in 0.1 N HCl solution as compared to solubility in distilled water. The Beer- Lambert's concentration range was found to be 4-20 μ g/ml at 290 nm and 260 nm for PYR and DOX respectively and coefficient of correlation were found 0.9999 and 1 (Table 1) for proposed method. Precision was determined by studying the interday and intraday precision. The standard deviation and Relative Standard deviation (% RSD) were calculated for both drugs. For proposed method % COV were not more than 1.0% which indicates good intermediate precision (Table 3 and 4). The values LOD and LOQ were 0.580 μ g/ml and 1.75 μ g/ml for PYR and 0.323 μ g/ml and 0.979 μ g/ml for DOX respectively (Table 5). Percentage estimation of PYR and DOX in tablet dosage form were 98.5 and 99.1 by the proposed method respectively (Table 6).

CONCLUSIONS

Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed method is new, simple, accurate, sensitive, economic and precise and can be adopted for routine analysis of drugs in tablet dosage form and as a tool to carry out in process quality monitoring to validate unit operations such as mixing time and granulation time.

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