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SECOND DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF DOXYLAMINE SUCCINATE AND PYRIDOXINE HCL IN PHARMACEUTICAL FORMULATIONS.

AMAR KUMAR KASTURI[1], KANAKA RAJU MEDICHERLA[2], RAGHUVEER YAGANTI[2] AND N. BALAKRISHNAN[3*]

[1]Department of Chemistry, Pacific University, Udaipur, Rajasthan-313003, India.
[2]Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh-530 003, India.
[3]Principal cum Professor, S A Raja Pharmacy College, Vadakkangulam-627116, Tamilnadu India.

ABSTRACT

Estimation of drugs in pharmaceutical formulations by using UV-Visible spectrophotometric method is one of the most frequently used techniques in pharmaceutical analysis. Simple and reliable second derivative spectrophotometric method was developed and validated for the simultaneous estimation of Doxylamine Succinate and Pyridoxine HCL in bulk and pharmaceutical formulations. The quantitative determination of the drugs was carried out using the second derivative values measured at 239 nm and 290 nm for Doxylamine Succinate and Pyridoxine Hcl respectively. The method has been developed and validated for the assay of Doxylamine Succinate and Pyridoxine HCL using methanol as diluents. Calibration graphs drawn at their wavelengths of determination were linear in the concentration range of 1-8 µg mL^{-1} for Doxylamine Succinate and 12.5 - 100 µg mL^{-1} for Pyridoxine Hcl. At 232 nm Doxylamine Succinate showed zero absorbance but Pyridoxine HCL had considerable amount of absorbance. Similarly at 290 nm Pyridoxine HCL showed zero absorbance but Doxylamine Succinate had considerable amount of absorbance. The limit of Quantification (LOQ) of Doxylamine Succinate and Pyridoxine HCL was found to be 0.6 µg mL^{-1} and 1µg mL^{-1} respectively and the limit of detection (LOD) was found to be 0.08 µg mL^{-1} and 0.3 µg mL^{-1} respectively. Comparison second derivative spectrum of Doxylamine Succinate and Pyridoxine HCL in standard and drug formulation solutions showed that the wavelength of maximum absorbance did not change. The low relative standard deviation values indicate good precision and high recovery values indicate accuracy of the proposed method. Developed second derivative spectrophotometric method was simple, accurate, precise, specific, sensitive and reproducible, which can be directly and easily applied to pharmaceutical dosage forms.

KEYWORDS: ***Spectrophotometric method, Second derivative, Doxylamine Succinate, Pyridoxine HCL.***

N. BALAKRISHNAN*
Principal cum Professor, S A Raja Pharmacy College, Vadakkangulam-627116, Tamilnadu India

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INTRODUCTION

Doxylamine Succinate Ethanamine, N,N-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]- butanedioate (Fig.1) Doxylamine is a first-generation antihistamine.[1] 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.[2-3] Doxylamine is also used in combination with the analgesics paracetamol (acetaminophen) and codeine as an analgesic/calmative preparation and is prescribed in combination with vitamin B6 (pyridoxine) to prevent morning sickness in pregnant women.[4-5] Pyridoxine HCL 4-pyridinedimethanol, 5-hydroxy-6-methyl hydrochloride (Fig. 2). It is a water soluble vitamin and involved principally in amino acid, carbohydrate and fat metabolism.[6-7]It is also required for the formation of haemoglobin.

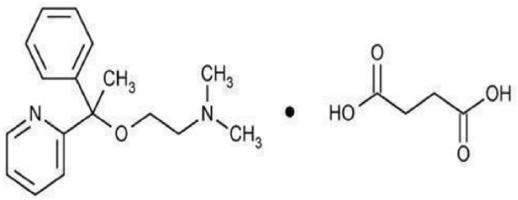

Fig. 1
Doxylamine Succinate.

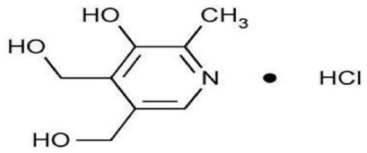

Fig. 2
Pyridoxine Hcl.

According to the literature survey it was found that few analytical methods such as Visible, UV, polarographic analysis and HPLC methods were reported for Pyridoxine Hcl and Doxylamine Succinate, but not even a single method has been reported on Second derivative spectroscopic method.[8-9] The objective of this study was to develop and validate a simple and specific Second derivative spectrophotometric method for the simultaneous determination of Doxylamine Succinate and Pyridoxine HCL in combined dosage form.[10-11] Derivative spectroscopy has been widely used as a tool for quantitative analysis.[12-13] This technique offers various advantages over the conventional spectrophotometric methods, such as discrimination of the sharp spectral features over the large bands and the enhancement of the resolution of the overlapping spectra.[14-15] This method exhibited a precise, accurate and cost effective assay for these drugs in mixture.

MATERIALS AND METHODS

Selection of Solvent

The derivative spectra[s] of Doxylamine Succinate and Pyridoxine HCL in different solvents like water, acetonitrile, sodium hydroxide and hydrochloric acid did not show any favorable zero crossing points, but when dissolved in methanol the derivative spectra[s] of both drugs showed zero crossing points. Hence methanol was selected as the solvent for the method.

Selection of derivative method

Though both first and second derivative spectra[s] showed zero crossing points in methanol solvent and their absorbance were considerably better, but the second derivative method was selected because the spectral characteristics and resolution were good in the second derivative spectra.

Selection of Wavelengths (Zero crossing points)

The zero crossing points of Doxylamine Succinate were 222, 232 and 235 nm and for Pyridoxine Hcl were 219, 239 and 241 nm. Out of these wavelengths 232 nm for Doxylamine Succinate and 239 nm for Pyridoxine Hcl were selected as the zero crossing points for the method based on their linearity data. At 232 nm Doxylamine Succinate showed zero absorbance but Pyridoxine Hcl had considerable amount of absorbance. Similarly at 239 nm Pyridoxine Hcl showed zero absorbance

but Doxylamine Succinate had considerable amount of absorbance.

Preparation of Standard stock solutions

Standard Doxylamine Succinate stock solution was prepared by dissolving 10mg of drug in 100 mL of methanol to get a concentration of 100 µg mL^{-1}. Standard Pyridoxine Hcl stock solution was prepared by dissolving 125 mg of drug in 100 mL of methanol to get a concentration of 1250 µg mL^{-1}. The standard solutions were prepared by dilution of the stock solution with methanol.

Chemicals

0.1N Hydrochloric acid, 0.1N Sodium hydroxide, Water, Acetonitrile, Methanol, Doxylamine Succinate and Pyridoxine HCL.

Instrument

Shimadzu UV-Visible spectrophotometer (model UV-1800).

***Validation Parameters*:**

Linearity

To construct Beer lambert's law plot for Doxylamine Succinate (1-8 mL) in different concentrations (12.5, 25, 37.5, 50, 62.5, 75, 87.5 and 100 µg/mL) and Pyridoxine Hcl (0.1 - 0.8 mL) in different concentrations (1, 2, 3, 4, 5, 6, 7 and 8 µg/mL), were prepared by serial dilutions with methanol from the individual stock solutions. Then absorbance of the solution was measured at 232 nm for Pyridoxine Hcl and 239 nm for Doxylamine Succinate. The standard overlain spectrum of Doxylamine Succinate and Pyridoxine Hcl was shown in Fig. 3. The linearity values were shown in Table 1.

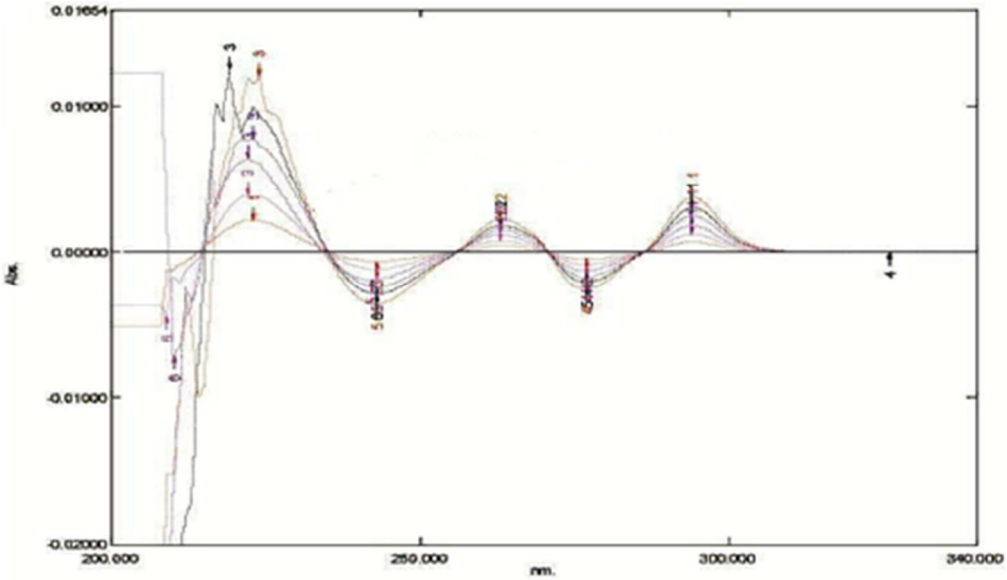

Fig.3

Overlain second derivative spectra of standard drugs (Doxylamine Succinate 2 µg mL^{-1} and Pyridoxine Hcl 25 µg mL^{-1}).

Table. 1

Linearity

Parameters	Doxylamine Succinate	Pyridoxine Hcl
Concentration (µg/ mL)	1-8	12.5-100
Slope	0.001	0.0001
Intercept	0.0001	0.0001
Correlation coefficient (r^2)	0.999	0.999

Recovery studies

The recovery studies were carried out at three different levels i.e. 80%, 100% and 120%. To ensure the reliability of the above method, recovery studies were carried out by mixing a known quantity of standard drug with the pre analyzed sample formulation and the contents were reanalyzed by the proposed method. The percentage recovery values were shown in Table. 2.

Table. 2
Recovery studies

Drug	Amount added	Amount recovered	% Recovery
Pyridoxine Hcl	12.5	12.47	99.76
	10	9.95	99.50
	15	14.94	99.60
DoxylamineHcl	1	0.991	99.10
	0.8	0.793	99.12
	1.2	1.19	99.16

Precision

The precision of the method was established by carrying out the analysis of the analytes using the proposed method. The low value of standard deviation showed that the methods were precise. The results were shown in Table. 3.

Table. 3
Precision studies

Drug	Concentration	Intraday concentration measured	% RSD	Interday Values	%RSD
Pyridoxine Hcl	25	25.06	0.641	25.08	0.962
Doxylamine Succinate	2	2.03	0.874	2.04	0.891

Specificity

Comparison second derivative spectrum of Doxylamine Succinate and Pyridoxine Hcl in standard and drug formulation solutions showed that the wavelength of maximum absorbance did not change. According to the results obtained by recovery study, the derivative spectrophotometric method is able to access the analyte in presence of excipients and hence it can be considered as specific method.

LOD and LOQ

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions. The LOQ of Doxylamine Succinate and Pyridoxine Hcl was found to be 0.6 µg mL^{-1} and 1µg mL^{-1} respectively and the LOD was found to be 0.08 µg mL^{-1} and 0.3 µg mL^{-1} respectively.

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of reproducibility of assay results Obtained by the successful applications of assay over different time, day and among multiple analysts. The results showed no statistical differences suggesting that the developed method was rugged.

Preparation of Test Solution and estimation of Pyridoxine HCl and Doxylamine Succinate in formulation

For analysis of commercial formulations, 20 capsules were weighed, and powdered and weight equivalent to 12.5 mg of Pyridoxine HCl and 1 mg of Doxylamine Succinate was taken and transferred into a volumetric flask and made up to 100 mL with methanol, sonicated for 5 minutes, filtered and further diluted with methanol to get the concentration within the linearity range of respective drugs and measured the absorbance at 232 nm for Pyridoxine HCl and 239 nm for Doxylamine Succinate respectively. The formulation spectrum shown in Fig. 4. Then the amount of drug present in the formulation was calculated and results were shown in Table 4.

Table. 4
Analysis of formulation

Drug	Amount labeled (mg/mL)	Amount claimed (mg/mL)	% Label Claim	% Deviation
Pyridoxine Hcl	5 mg	4.95	99.00	(-) 1.0
Doxylamine Succinate	0.4 mg	0.397	99.25	(-) 0.75

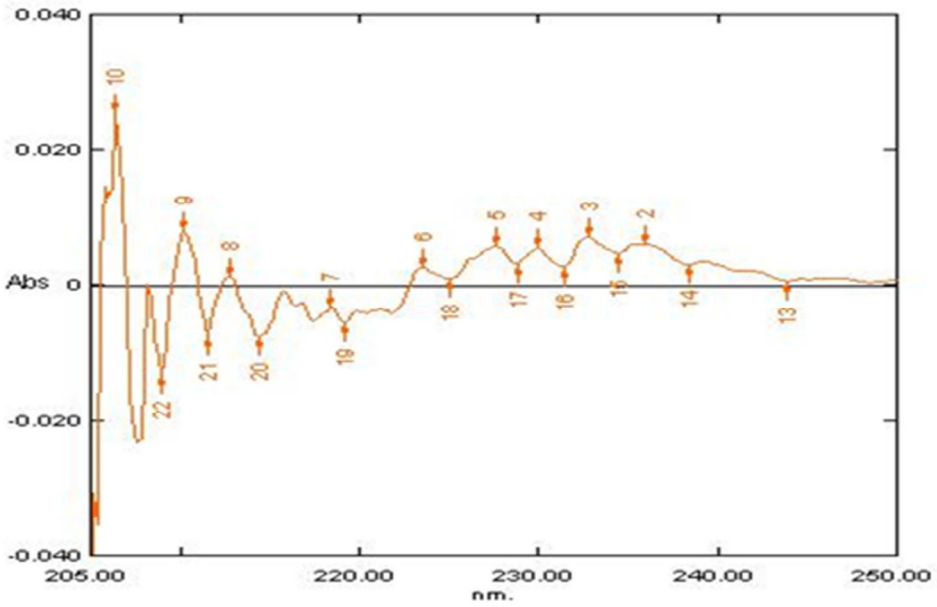

Fig. 4
Second derivative spectra of formulation (Doxylamine Succinate 2 µg mL and Pyridoxine Hcl 25 µg mL)

RESULTS AND DISCUSSION

From the optical characteristics obtained with the proposed method it was found that the drug obeys linearity with in concentration range of 12.5 - 100 µg mL for Pyridoxine Hcl and 1-10 µg / mL for Doxylamine Succinate. From the precision studies, it was found that the percent relative standard deviation (% RSD) is less than 2%, which indicates that the method has good reproducibility. From the results of recovery studies, it was found that the percent recovery values of pure drug from the pre-analyzed solutions of formulations were in between 98.0-99.8%, which indicates the method is accurate and reveals that commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method. The proposed method was simple, sensitive and reliable with good precision and accuracy. Hence, this method can be used for the routine analysis of Pyridoxine Hcl and Doxylamine Succinate in bulk samples and pharmaceutical formulations. Results and discussion part should be elaborated. Graph and tables, Please ensure that it should also come in results and discussion, Please rewrite this part

CONCLUSION

A convenient and rapid UV method has been developed for simultaneous estimation of Pyridoxine Hcl and Doxylamine Succinate in available dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and interday % RSD coupled with excellent recoveries. Hence, this method can be easily and conveniently adopted for routine analysis of Pyridoxine Hcl and Doxylamine Succinate in pure form and its dosage forms.

AUTHOR'S CONTRIBUTION STATEMENT

Conceived and designed the experiments: AKK, NB. Performed the experiments: AKK, KM. Analyzed the data: AKK, NB. Contributed reagents/materials/analysis tools: NB, RY. Wrote the paper: AKK, KM.

CONFLICT OF INTEREST

Conflict of interest declared none

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