

## Research Article



## Method Development and Validation for the Simultaneous Estimation of Amiodarone and Propranolol by Using UV Spectroscopy

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### ABSTRACT

This research work aims to develop simple, accurate, and precise analytical methods for the simultaneous estimation of Propranolol Hydrochloride and Amiodarone Hydrochloride, used for the treatment of cardiovascular diseases. The UV-visible spectrophotometric method was established for the estimation. Amiodarone Hydrochloride show better solubility in methanol and the solubility of propranolol Hydrochloride in methanol is increased by the addition of DMSO (Dimethyl sulfoxide). UV-visible spectrophotometric methods showed linearity within the range of 16-24 µg/ml for propranolol and 4 -8µg/ml for Amiodarone. λ<sub>max</sub> were 288 nm and 242nm respectively. The percentage recoveries of Propranolol and Amiodarone were 100% and 98.6% respectively. The correlation coefficient was found to be 0.998 for Propranolol and 0.999 for Amiodarone. The UV-visible spectroscopic method is rapid, cost-effective, more precise, and accurate. This method can be useful since no analytical method was developed for Propranolol and Amiodarone in combination.

**Keywords:** Propranolol, Amiodarone, method development, Vierordt's method, validation.

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### INTRODUCTION

Cardiovascular disease is a class of diseases that are related to the heart or blood vessels including stroke, heart failure, hypertension, coronary artery diseases, heart arrhythmia, peripheral artery disease, and atherosclerosis<sup>1,2</sup>. Atherosclerosis is one of these conditions which is caused by a build-up of plaque in a person's artery, this builds up can accumulate to the point that clot forms and clogs the artery completely, leading to either a stroke or a heart attack. When atherosclerosis occurs in the heart it is called coronary artery disease<sup>3,4</sup>.

Propranolol is a white powder, colourless, bitter in taste, it is soluble in ethanol and water practically insoluble in ether benzene and ethyl acetate<sup>5</sup>. Propranolol HCl is a non-selective β adrenergic blocking agent<sup>6</sup>. Propranolol HCl inhibits the response to adrenergic stimuli by completely blocking beta-adrenergic receptors within the myocardium and bronchial and smooth muscles<sup>7</sup>. Amiodarone hydrochloride is an iodinated benzofuran, a derivative antiarrhythmic agent<sup>8</sup>. The drug differs structurally and pharmacologically from another currently available antiarrhythmic agent. It is an important drug for the treatment of supraventricular and ventricular arrhythmias, in short-term inpatient and long-term outpatient settings<sup>9</sup>.

Analytical method development is the key element of the pharmaceutical development program. And it is the process of proving that the developed method can be used to detect the amount or concentration of API in various formulations. UV-visible spectrometry is one of the oldest instrumental techniques of analysis and is the basis for several ideal methods for the determination of micro and semi-micro quantities of analytes in a sample. The specific aim of the research work was to develop and validate the UV spectrophotometric method for simultaneous determination of Propranolol and Amiodarone<sup>10</sup>.

### Method Development by UV-Visible Spectrometer

Absorption spectroscopy is one of the preferred and widely used tools available for quantitative analysis. The relation between the concentration of analyte and the amount of light absorbed is the basis of most analytical applications of molecular spectroscopy. This review establishes the fundamentals of following simultaneous spectrometric estimation methods which are employed for quantitative estimation of multi-component formulations.

### Simultaneous Equation Method (Vierordt's Method)<sup>11-15</sup>

One of the most common and easiest methods employed for spectrophotometric multicomponent analysis is that the concentration of several components present in the given mixture can be determined by solving a set of simultaneous equations even if their spectra overlap. This method derives its principle from the additive nature of absorbance of individual components in any mixture.

Suppose a sample contains two absorbing species X and Y. Absorbance of this mixture is measured at wavelengths of



maximum absorbance ( $\lambda_{max}$ ) of each drug ( $\lambda_1$  for drug Y and  $\lambda_2$  for drug X)

Thus, the individual concentrations of both drugs may be determined by using the simultaneous equation method provided that certain criteria are fulfilled. The information required is:

$a_{x1}$  and  $a_{x2}$ , being the absorptivity of drug X at  $\lambda_1$  and  $\lambda_2$  respectively.

$a_{y1}$  and  $a_{y2}$ , being the absorptivity of drug Y at  $\lambda_1$  and  $\lambda_2$  respectively.

$A_1$  and  $A_2$ , are the absorbance of the diluted sample at  $\lambda_1$  and  $\lambda_2$  respectively.

Let  $C_x$  and  $C_y$  be the concentrations of drug X and drug Y respectively in the diluted samples. At  $\lambda_1$  and  $\lambda_2$ , the absorbance of the mixture in a constant path length  $b$  is the sum of the individual absorbance of X and Y. Simultaneous equations are thereby constructed.

$$\text{At } \lambda_1; \quad A_1 = a_{x1}bC_x + a_{y1} \dots \dots \dots (1)$$

$$\text{At } \lambda_2; \quad A_2 = a_{x2}bC_x + a_{y2} \dots \dots \dots (2)$$

For measurements in 1 cm cells,  $b = 1$

By Cramer's rule,  $C_x$  and  $C_y$  can be determined as follows

$$C_x = (A_2 a_{y1} - A_1 a_{y2}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$

$$C_y = (A_1 a_{x2} - A_2 a_{x1}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$

## MATERIALS AND METHODS

### Chemicals and reagents

Propranolol Hydrochloride and Amiodarone Hydrochloride were purchased from Yarrow Chem Chemicals, Mumbai. Methanol, Dimethyl Sulfoxide, was purchased from Mumbai's Research lab's fine chemicals industries. Distilled water was obtained from the local market.

### Equipments

Electronic balance- Tandem TJ series

UV spectrophotometer- Shimadzu, UV1700, Japan which is attached to a computer software UV probe 2.0 with a spectral width of 2 nm, wavelength accuracy of 0.5 nm, and a pair of 1cm matched quartz cell

### Simultaneous estimation of propranolol Hydrochloride and Amiodarone Hydrochloride using simultaneous equation method (Vierordt's Method)

#### Selection of wavelength range for estimation

Both propranolol and Amiodarone were dissolved separately in methanol and appropriate dilutions were prepared by taking aliquots from the stock solution. To increase the solubility of propranolol in methanol a drop of DMSO is added. The drug solutions were scanned from 200-400nm and from that wavelength ranges are selected for the estimation of drugs.

### Preparation of standard stock solutions (1000µg/ml)

From the above-prepared stock solutions of propranolol hydrochloride and amiodarone, hydrochloride 1ml was transferred separately to a 10ml volumetric flask to obtain working standard solutions having a concentration of 100µg / ml

### Preparation of calibration curve

From the above working standard solutions of both propranolol (1.6,1.8,2.0,2.2 and 2.4 ml) and Amiodarone (0.4,0.6,0.7 and 0.8 ml), aliquots were transferred separately in a series of 10 ml volumetric flask. The volume was adjusted to the mark with methanol. To get a concentration range of 16-24µg / ml of Amiodarone hydrochloride. All solutions' absorbance was calculated by scanning from 200-400 nm, against methanol as the blank.

### Methodology

The working standard solutions of Propranolol and Amiodarone were scanned in UV from the range of 200-400 nm, where propranolol shows 288 and Amiodarone shows 242 nm as the wavelength having maximum absorbance, and this wavelength is selected for the quantitative estimation of propranolol and amiodarone. Hence, the concentration of the two components was calculated by the technique of simultaneous equation (Vierordt's method).

### Method validation:

#### Linearity

The linearity of the method was checked in the concentration range of 16-24µg / ml for propranolol and 4-8µg / ml of Amiodarone. The calibration curves were constructed by plotting the graph of absorbance vs concentration. A linear regression equation was obtained over the concentration range ( $y = mx+c$ )

#### Precision

The precision of the method was accessed by repeated scanning and measuring the absorbance of propranolol (20µg / ml) and Amiodarone (6µg / ml) without changing the parameters of the proposed methods 6 times( $n=6$ )

#### Intermediate precision (reproducibility)

The intraday and interday precision of the proposed methods was determined by analyzing the responses 3 times on the same day and on 3 different days over a period of 1 week for 3 standard solutions of PROP ( 20, 22 and 24 µg/ml) and AMIO (6, 7 and 8 µg/ml). The results are reported in terms of standard deviation and percent Relative Standard Deviation (% RSD).

#### Accuracy

The accuracy of the method was determined by calculating the recovery of propranolol and amiodarone by the standard addition method. The known amount of standard solutions of propranolol and amiodarone were added at 80,100,120 % level to pre-quantified sample solutions of



Propranolol (20µg / ml) and Amiodarone (6µg / ml). The amounts of Propranolol and Amiodarone were estimated by applying the obtained values to the regression equation of the calibration curve.

**Limit of detection and Limit of quantification**

The limit of detection and the limit of quantification of the drug was derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where,  $\sigma$  = the standard deviation of the response

S = slope of the calibration curve

**Range**

The range of an analyte's highest and lowest concentrations is referred to as the range that demonstrate the appropriate degree of precision, accuracy, and linearity of the analytical procedure. PROP

(16–24 g/ml) and AMIO (4–8 g/ml) concentration ranges were used to determine the method's range. A precisely measured standard working solution of PROP and AMIO was constructed for the evaluation of the range.

**Ruggedness**

Six injections of a sample solution were made to assess the ruggedness. The system under examination was subjected to two rounds of analysis using two distinct analysts. The results of the test were confirmed to be within acceptable bounds, and the % RSD value was less than 2.

**RESULT**

The UV-Visible spectrophotometric method was developed to simultaneously estimate Propranolol Hydrochloride and Amiodarone Hydrochloride in bulk and formulation. In this method, the dilute solution of Propranolol and Amiodarone was scanned from 200-400 nm. Two wavelengths 288nm and 242nm are selected from Propranolol Hydrochloride and Amiodarone Hydrochloride respectively. Here 288 nm is the absorbance maxima of PROP and 242 nm is the  $\lambda$  max of AMIO.

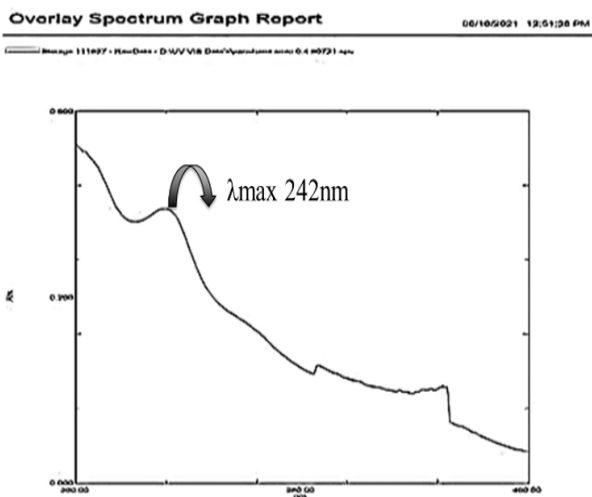


Figure 1: UV spectrum of Amiodarone Hydrochloride

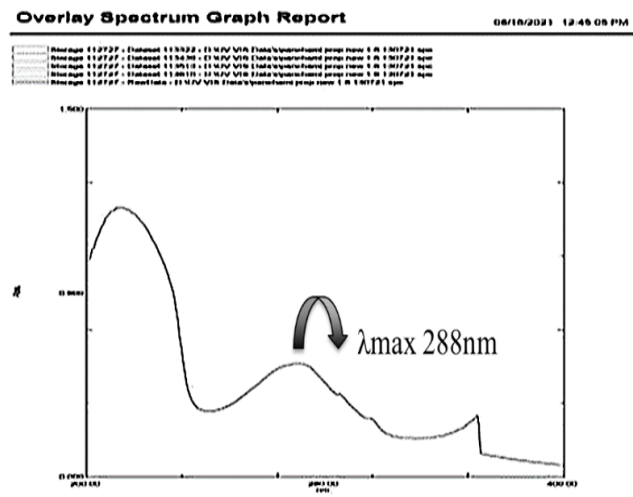


Figure 2: UV spectrum of Propranolol Hydrochloride

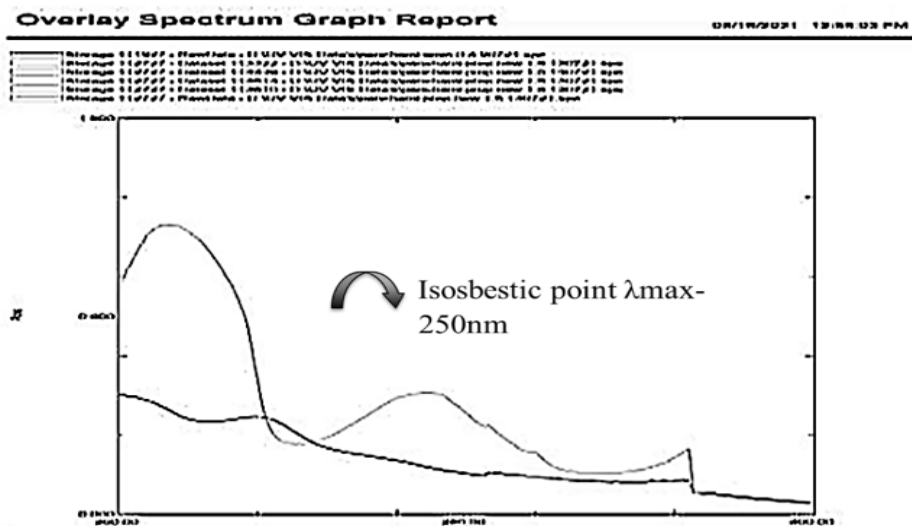
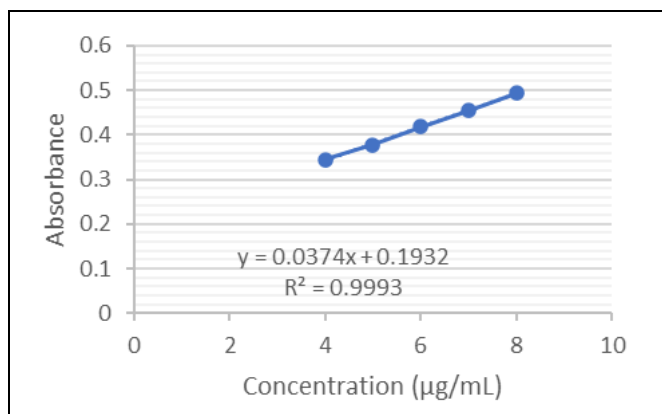
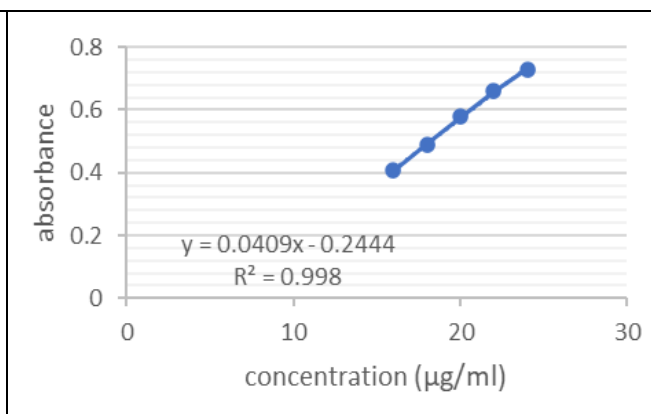


Figure 3: Overlay spectrum of Amiodarone Hydrochloride and Propranolol Hydrochloride





**Figure 4:** Calibration curve of Amiodarone Hydrochloride



**Figure 5:** Calibration curve of Propranolol Hydrochloride

**DISCUSSION**

**Table 1:** Result of repeatability

Concentration (n=6)	Absorbance	
	Propranolol (288nm) 20µg/mL	Amiodarone (242nm) 6µg/mL
1	0.592	0.414
2	0.591	0.413
3	0.590	0.412
4	0.592	0.414
5	0.591	0.414
6	0.592	0.413
<b>Mean</b>	0.591333	0.413333
<b>SD</b>	0.000816	0.000816
<b>RSD (%)</b>	0.137	0.197

**Table 2:** Result of Accuracy

Drug	Accuracy Level (%)	Actual Amount (µg/mL)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean±SD	%RSD
Propranolol Hydrochloride	80%	20	16	36	98.81	99.45±0.600	0.603
	100%	20	20	40	100		
	120%	20	24	44	99.54		
Amiodarone Hydrochloride	80%	6	4.8	10.8	98.14	99.12±0.935	0.943
	100%	6	6.0	12	100		
	120%	6	7.2	13.2	99.24		

**Table 3:** Validation data

Parameters	Propranolol Hydrochloride	Amiodarone Hydrochloride
Linearity range (µg/mL)	16-24	4-8
Correlation coefficient (r)	0.998	0.999
Precision (%RSD)	0.137	0.197
Accuracy (%RSD)	0.603	0.943
Limit of detection (µg/mL)	0.54	0.157
Limit of quantification (µg/mL)	1.64	0.478



**CONCLUSION**

The UV-Spectrophotometric method was developed and validated as per ICH guidelines. The developed method was more simple, accurate, precise, and economic as compared to other spectrophotometric methods, thus can be used routinely for the analysis of propranolol and amiodarone in the bulk and combined dosage forms.

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