Research Article

# Application of Newly Developed and Validated Analytical Method for Interaction Study of Selected Cardiac Drug Under Clinical Trials for Combination Therapy

**Dr. Prasanth S.S[3], Panchami G.S[2], Blessy Thomas[1]*, Reeba K.R[4]**

[3] Research center by Shifa medical trust, Al Shifa College of pharmacy Kizhattur, Malappuram, Kerala, India.

[1,2,4] Department of Pharmaceutical Analysis Al Shifa College of Pharmacy, Poonthavanam p.o, kizhattur, Malappuram, Kerala, India

***Corresponding author's E-mail:** thomasblessy1998@gmail.com

* **ORCID id –** 0000-0003-3527-5429

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**ABSTRACT**

This research work aims to develop a new, rapid, accurate, and precise FTIR method for the simultaneous estimation of Propranolol Hydrochloride and Amiodarone Hydrochloride. are used for the treatment of cardiovascular diseases and application of the developed FTIR method to determine interaction by closely observing their interactive peaks. Acc. to WHO, Cardiovascular diseases are a group of disorders of the heart and blood vessels. As per statistics, about 1.7 million deaths are reported yearly due to CVD. The developed method was validated according to ICH guidelines. Amiodarone Hydrochloride shows better solubility in methanol and the solubility of Propranolol Hydrochloride in methanol is increased by the addition of DMSO (Dimethyl sulfoxide). Sample preparation was not required since ATR method were used. For the drugs, FTIR Beer's-Lambert's law was obeyed in the concentration range of 0.5-5.0 mg and 3.8-10.3mg respectively. 96.6% and 98.59% were the percentage recovery of the drugs respectively for FTIR. Correlation coefficient was found to be 0.996 for Propranolol and 0.999 for Amiodarone. FTIR. This method can be useful since no analytical method was developed for Propranolol and Amiodarone in combination. The applicability of the developed method was determined by carrying out interaction studies and concluded by a positive result of no physical interaction between both.

**Keywords:** Propranolol Hydrochloride, Amiodarone Hydrochloride, FTIR, ICH Validation guidelines, drug interaction, Simultaneous method development.

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## INTRODUCTION

### Cardiovascular disease

Cardiovascular diseases [1] are a collection of diseases that affect the heart and blood vessels of the human body like coronary heart disease[2], angina[3], stroke[4], rheumatic heart disease[5], congenital heart disease[6], peripheral arterial disease[7], aortic aneurysm[8] and dissection, deep vein thrombosis[9] etc.

### Propranolol

Propranolol[10] is a Beta-adrenergic blocker that increases collateral blood flow and redistributes blood to ischemic areas. It has an equal affinity for β1 and β2-receptors, lacks intrinsic sympathomimetic activity (ISA)[11] and has no α-adrenergic receptor activity. Propranolol improves microcirculatory oxygen delivery thus oxygen dissociates more easily from haemoglobin after the β-adrenergic blockade. Platelet aggregation is also inhibited. It is a lipid-soluble β-blocker and mostly possesses central nervous system side effects. 90% of the drug is metabolized at First-pass liver metabolism[12] so requires a higher dose during oral administration compared to intravenous doses for the pharmacodynamics effect. A common intravenous dose of propranolol initially is 0.5 to 1.0 mg titrated to effect. A continuous infusion of 1 to 3 mg/hr. can prevent hypertension and tachycardia but to be used cautiously because of the potential chances of cumulative effects. [13]

### Amiodarone

Amiodarone has emerged as an important Class III antiarrhythmic drug[14] which prolongs the duration of action potential in cardiac muscle that mediates repolarization by blocking the potassium channel and thus increases the refractory periods of cardiac tissue [15]. It has both antiarrhythmic and potent vasodilator activity. The antiarrhythmic effect [16] of amiodarone is due to two major reasons, prolongation of cell-action potential [17] and refractory period [18] of myocardial cells second a non-competitive αand β- adrenergic inhibition. Amiodarone has a very slow onset of action (days) with a half-life of approximately a month following oral doses thus being eliminated from the body very slowly. This drug has iodine atoms in its nucleus therefore it affects thyroid hormones. Hypothyroidism [19] is observed in 11% of patients. The acute effects of intravenous amiodarone administration are predominantly, β-receptor, calcium channel, and sodium-channel blockade. The class III [20] effect is observed after completion of the loading dose due to levels of the active metabolite desethylamiodarone. For Class III drugs reverse use dependence has been coined. Reverse use

dependence means that at slower heart rates, the prolongation of the action potential is most pronounced at faster heart rates, and the effect diminishes. This is related to a drug 's binding characteristics. Drugs that preferentially bind with closed potassium channels show significant reverse use dependence because phase 4 of the action potential is longer when the heart rate is slow. [21]

## MATERIALS AND EQUIPMENT

### Materials and reagent

Propranolol Hydrochloride and Amiodarone Hydrochloride were purchased from Yarrow chem chemicals Hyderabad. Methanol, Dimethyl sulfoxide was purchased from nice chemicals by the institution.

### Equipment

FTIR of Bruker ATR, and Alpha interferometer attached to OPUS Software were used throughout the analysis.

## EXPERIMENTAL METHODOLOGY

### Sample Preparation

No sample treatment is required for FTIR [22] except grinding. Here, the powdered sample is introduced on the top of ATR crystal, and spectra were recorded between 4000 and 650cm$^{1}$, by averaging 24 scans for spectrum using OPUS software of Bruker-α /ZnSe FTIR spectrophotometer with Reflection Top-Plate. A pressure plate and clamp were used to compress the sample against the crystal.

### Identification of Drugs by FTIR Spectroscopy

FTIR was scanned from 400-4000 cm$^{-1}$. Spectrum was used for the identification of drugs.

#### *Propranolol hydrochloride*

Accurately weighed 100mg pure drug of Propranolol and placed on top of ATR crystal and spectra were recorded between 4000 and 650 cm$^{-1}$, and taking an average of 24 scans for spectrum using OPUS software of Bruker-α /ZnSe FTIR spectrophotometer with Reflection Top-Plate. A pressure plate and clamp were used to compress the sample against the crystal. One isolated peak 3421.89-3134.14cm$^{-1}$ was defined and the peak integral area was calculated noted in table 1

#### *Amiodarone hydrochloride*

Accurately weighed 100mg pure drug and placed on top of ATR crystal and spectra were recorded between 4000 and 650 cm$^{-1}$, by averaging 24 scans for spectrum using OPUS software of Bruker-α /ZnSe FTIR spectrophotometer with Reflection Top Plate. A pressure plate and clamp were used to compress the sample against the crystal. One isolated peak 2454.01-2362.45 cm-1 was defined and the peak integral area was calculated. And noted in table 1.

### Method validation

#### *1 Accuracy*

The method's accuracy was expressed in percentage recovery and is calculated by the standard addition technique. Here the percentage spiking levels are 80,100 and 120 percent. The result obtained was recorded in Tables 2 and 3.

#### *2 Method precision (Repeatability)*

The precision of the instrument was checked by repeated scanning and measuring the absorbance of the solution of (n=6) Propranolol (3mg/ml) and Amiodarone (7mg/ml) without changing the parameters of the developed methods. Repeatability was noted in table 2

#### *3 Reproducibility*

The intraday and interday precision was determined by analyzing the corresponding responses 3 times on the same day and 3 different days over 1 week for 3 different concentrations of standard solutions of Propranolol (5,4,3mg/ml) and Amiodarone (7,5,4mg/ml). Relative standard deviation (% RSD) was used to report the results and was noted in table 5.

#### *4 Limit of detection and Limit of quantification (LOD & LOQ)*

The LOD and LOQ were calculated by the equation method shown in Table 2.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

#### *5 Linearity*

Different weights of Propranolol ranging from 0.5-5.0 mg and Amiodarone from 3.8-10.3 mg were taken using an electronic balance with 0.001 mg sensitivity and spectrum recorded and an average of such three determinations was plotted in a calibration curve Linearity was established by regression analysis and the correlation coefficient was determined and reported in table 3.

#### *Applicability of the Developed Method*

The two drugs Amiodarone and Propranolol Hydrochloride were mixed externally in a ratio of 3:7 to prepare the admixture. The admixture was scanned from 400-650cm$^{-1}$. Compare the active peaks of admixture and single drug peaks noted for the changes and result were noted.

## RESULTS AND DISCUSSION

### Identification of drug

**Table 1:** Analysis of drugs in admixture

| Admixture | Drug | Sample concentration (µg/ml) | Amount found (µg/ml) | Drug content (%) ±SD |
|---|---|---|---|---|
| 1 | Propranolol. Hydrochloride | 3.0 | 2.9 | 96.6 |
| | Amiodarone Hydrochloride | 7.3 | 7.1 | 98.59 |

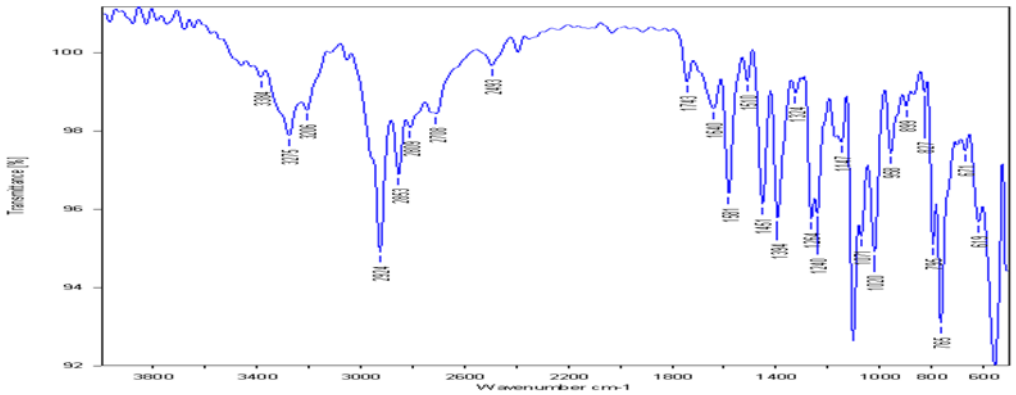


**Figure 1:** FTIR spectrum of Propranolol Hydrochloride

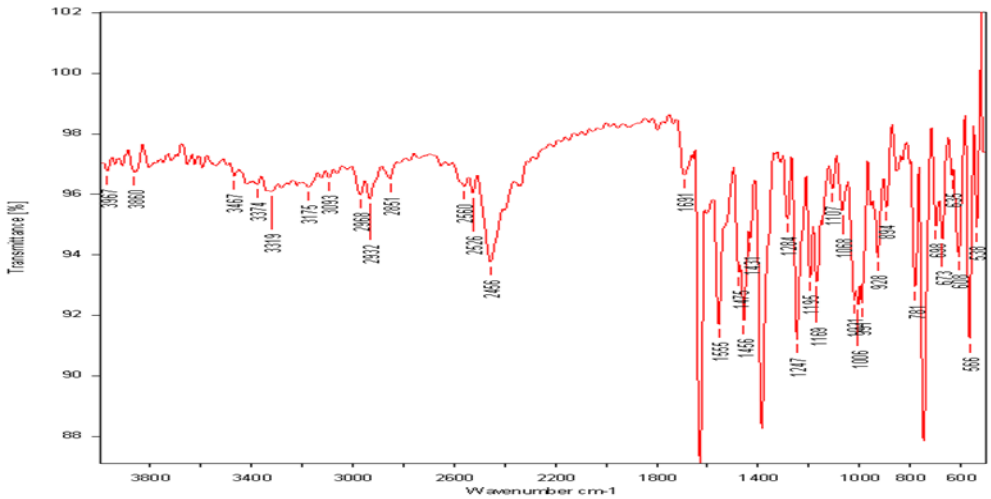


**Figure 2:** FTIR spectrum of Amiodarone Hydrochloride

### *Method Validation*

**Table 2:** Method Validation acc. to ICH Guidelines.

| Validation parameters | Propranolol Hydrochloride | Amiodarone Hydrochloride |
|---|---|---|
| Linearity | 0.5-5.0 mg | 3.8-10.3 mg |
| Correlation coefficient | 0.996 | 0.999 |
| % Recovery | 98.75 ±0.936 | 99.3 ±0.655 |
| Accuracy (%RSD) | 0.94 | 0.659 |
| Precision (%RSD) | 0.436 | 0.127 |
| Limit of detection | 0.47µg/mL | 2.9µg/mL |
| Limit of quantification | 0.49µg/mL | 3.8 µg/mL |

***Linearity***

**Table 3:** Determination of linearity

| Parameters | Propranolol Hydrochloride | Amiodarone Hydrochloride |
|---|---|---|
| Beer's range | 0.5- 5.0 mg | 3.8-10.3 mg |
| Correlation coefficient | 0.996 | 0.999 |
| Regression equation | Y=3.5958-1.962X | Y=201.69-0.56869X |
| Slope | 1.962 | 0.01120 |
| Y Intercept | 3.5958 | 0.01120 |

***Accuracy***

**Table 4:** Determination of accuracy

| Drug | Accuracy level (%) | Actual amount (mg) | Amount added (mg) | Amount found (mg) | %Recovery | Mean± SD | %RSD |
|---|---|---|---|---|---|---|---|
| Propranolol. Hydrochloride | 80% | 3 | 2.4 | 5.20 | 98.11 | 98.75± 0.936 | 0.94 |
| | 100% | 3 | 3.0 | 5.99 | 99.33 | | |
| | 120% | 3 | 3.6 | 6.49 | 98.33 | | |
| Amiodarone Hydrochloride | 80% | 7 | 5.6 | 12.5 | 99.20 | 99.30± 0.655 | 0.659 |
| | 100% | 7 | 7 | 14.0 | 100 | | |
| | 120% | 7 | 8.4 | 15.2 | 98.70 | | |

***Precision***

The percentage RSD of the Propranolol hydrochloride 3mg (n=6) was found to be 0.436 and Amiodarone Hydrochloride 7mg (n=6) was 0.127 was within the limit as per ICH guidelines.

***Reproducibility***

**Table 5:** Determination of reproducibility

| Drug (n=3) | Concentration (mg) | Intraday Amount found (mg) | | Interday Amount found (mg) | |
|---|---|---|---|---|---|
| | | Mean ±SD | %RSD | Mean ±SD | %RSD |
| Propranolol. Hydrochloride | 5 | 4.86±0.057 | 1.172 | 4.93±0.057 | 1.15 |
| | 4 | 3.99±0.005 | 0.125 | 3.80±0.057 | 1.50 |
| | 3 | 2.99±0.005 | 0.165 | 2.96±0.051 | 1.72 |
| Amiodarone. Hydrochloride | 7 | 6.93±0.057 | 0.826 | 6.96±0.057 | 0.816 |
| | 5 | 4.93±0.057 | 1.15 | 4.96±0.057 | 1.140 |
| | 4 | 3.93±0.057 | 1.45 | 3.86±0.057 | 1.470 |

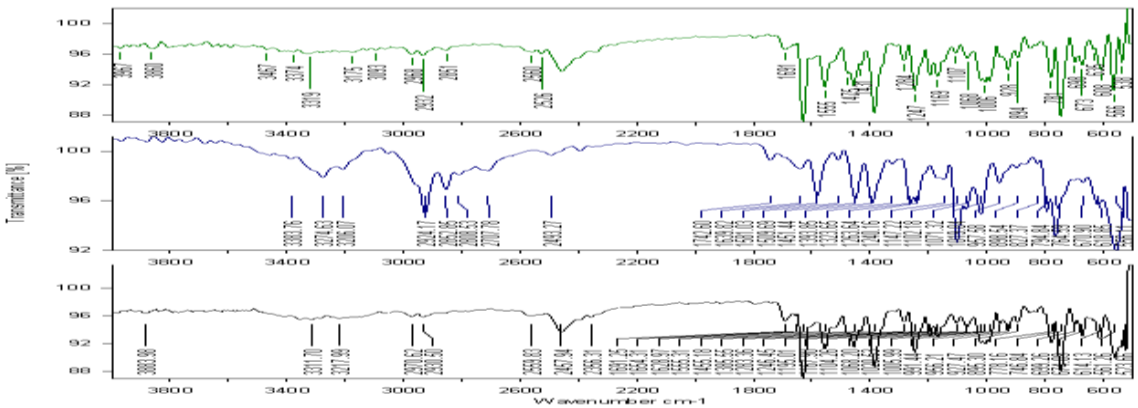


**Figure 3:** FTIR interaction spectrum. 1) spectrum of Amiodarone Hydrochloride. 2) Spectrum of Propranolol Hydrochloride. 3) Spectrum of admixture.

**Application of Method Developed**

Using the developed FTIR method the physical interaction between Amiodarone hydrochloride and Propranolol Hydrochloride were studied and shown in fig. 3. There were no deletions or changes in the active peaks of both drugs were observed in the admixture. It indicates no physical interaction between both. This preliminary reference data can in clinical trials and formulation development.

## CONCLUSION

A new simple, rapid, and precise analytical spectrophotometric FTIR method was developed to simultaneously determine Propranolol Hydrochloride and Amiodarone Hydrochloride. The method fulfilled validation requirements acc. to ICH guidelines. The developed method obeys Beer's Lambert's law over a concentration range of 0.5-5.0 mg for Propranolol Hydrochloride and 0.5-5.0 mg for Amiodarone Hydrochloride. Both drugs show a correlation coefficient of 0.996 and 0.999 respectively. No changes in the active peak of both drugs were observed in admixture using the developed FTIR method indicating no physical interaction between these drugs. The FTIR method developed reduces solvent consumption and eliminates the usage of reagents.

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