

## Effect of solvents on the Fluorescent spectroscopy of Riociguat

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### ABSTRACT

The influence of solvents on fluorescence emission spectra has been widely studied in various solvents, viz. Water, Dimethyl sulfoxide (DMSO), Dimethylformamide (DMF), Acetonitrile (ACN), Methanol (MEOH), Dichloromethane (DCM). An Increase in solvent polarity results in a decrease in fluorescence intensity. The fluorescence intensity was observed in between 220 to 1015 (a.u). The excited state dipole moments are observed to be greater than their ground state counterparts in all the solvents studied.

**Keywords:** Riociguat, Fluorescence Emission, Solvent Polarity, Stokes shift, Intensity

### I. INTRODUCTION

Chemically Riociguat is methyl 4, 6-diamino-2-[1-(2-fluorobenzyl)-1H-pyrazolo [3, 4-b] pyridine-3-yl]-5-yl-N- methylcarbamate. It's sold under the brand name Adempas. Commonly observed adverse effects of drug include dizziness, headache, peripheral edema, nausea, vomiting, diarrhoea, dyspepsia, nasopharyngitis & significant occurrence of hypotension was reported<sup>1</sup>. Riociguat is a novel soluble guanylate cyclase Stimulator it's used to treat chronic thromboembolic pulmonary hypertension<sup>2</sup>. This drug directly activates soluble guanylate cyclase (sGC) in a manner Independent of nitric oxide, thereby enhancing the enzyme's sensitivity to nitric oxide and promoting an increase in cGMP production & thereby resulting in vasorelaxation<sup>3</sup>.

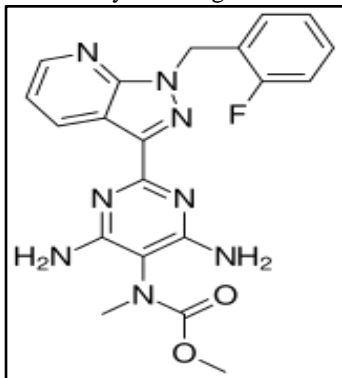


Fig 1: Chemical structure of Riociguat

The drug has received its first global approval on the 23<sup>rd</sup> of September 2013 to treat chronic thromboembolic pulmonary Hypertension (CTEPH). Riociguat is available only in film coated tablet dosage form<sup>4</sup>.

From the literature survey it has been evident that there are several methods reported viz. comparative spectrophotometric, spectrofluorimetric, HPLC, LC-ms/ms but study focused on Effect of solvent using spectrofluorimetry has not reported. Additionally, spectrofluorimetry offers advantages such as lower cost as compared to chromatography and ease of instrument handling<sup>5</sup>.

Main aim of the present work is to examine the effect of the solvents such as water, DMSO, DMF, ACN, MEOH, and DCM<sup>6</sup>. Solvent polarity significantly influences the spectral emission properties of the fluorophore molecules<sup>7</sup>. The fluorescence emission spectra of numerous fluorophore are influenced by the polarity of their surrounding environment<sup>8</sup>. The fluorescence spectral characteristics, including emission intensity, stokes shift, and maximum emission wavelength were significantly influenced by the type of the solvent used<sup>9</sup>.

### II. MATERIAL AND METHODS

#### Materials

Generous gift sample of Riociguat was procured from one of the reputed company ALEMBIC PHARMACEUTICAL, Vadodara. Solvents used were of analytical grade (Merck, India).

#### Instrumentation

Spectrofluorimeter Model RF-5301PC (Shimadzu) with xenon arc (150 w) lamp along with quartz cells 48 mm height, 10 mm path length with 0.5 mm slit width. Weighing balance (Vibra HT, Essae) was used for weighing the Riociguat.

## Methods

### Preparation of standard stock solution

Accurately weigh 1 mg of Riociguat and dilute it with 1ml (i.e. 1mg/ml) of suitable solvent viz Methanol, Acetonitrile, Dimethyl sulphoxide, Dimethyl formamide, Dichloromethane, water.

### Preparation of working solution

From the above working standard solution of 1000 ug/ml solution was prepared in different

solvents and further it was diluted to 10 ug/ml, 1000 ng/ml, 100 ng/ml, and 10ng/ml in different solvent. Final dilution of 10 ng/ml was scanned to check the fluorescence intensity as well as emission maxima.

### Preparation of calibration curve

Five working standard solutions with a concentration of 2, 4, 6, 8, and 10ng/ml has been prepared in DMF Solvent.

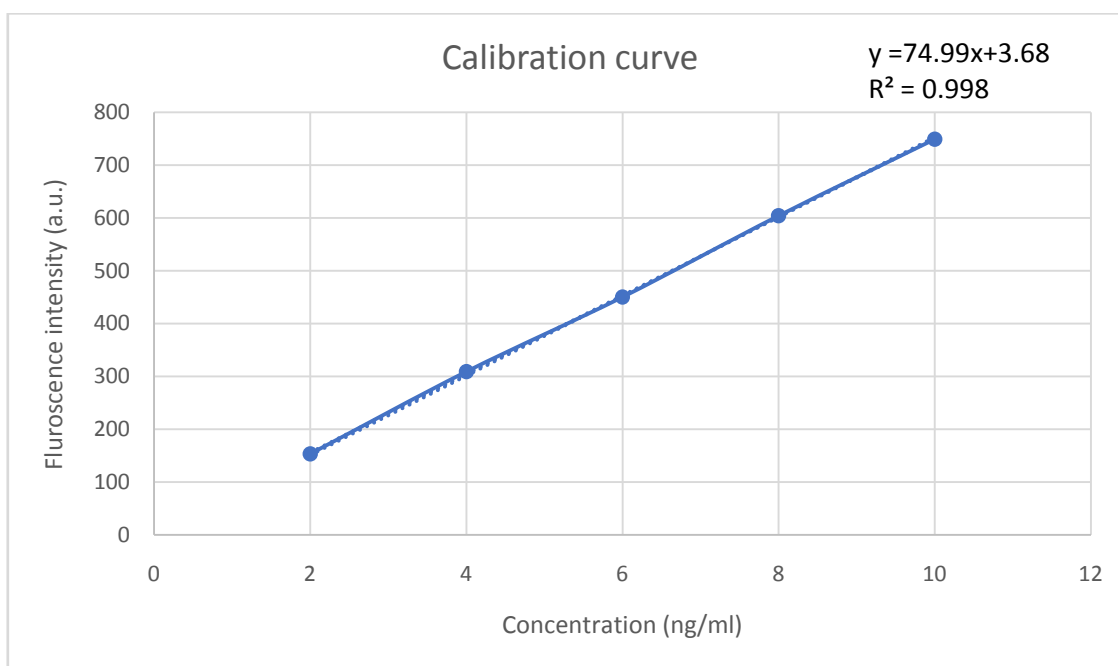


Fig 2: Calibration curve of Riociguat

## III. RESULT AND DISCUSSION

### Solvent effect on Fluorescence Emission

The effect of different solvents on the fluorescence emission intensity of Riociguat was investigated using the spectrofluorimetry. The solvents selected varied in polarity and hydrogen bonding capacity including water, Dimethyl sulphoxide, Dimethyl formamide, Acetonitrile, Methanol, and Dichloromethane. The fluorescence intensity data is summarized in Table 1 and figure 3.

Upon analysis, it was observed that Riociguat exhibited nearly identical fluorescence intensities in DMSO and ACN. This similarity suggests that the fluorescence of Riociguat is relatively insensitive to change in solvent polarity among polar aprotic solvents, such behaviour can be attributed to the rigid aromatic structure of Riociguat and the minimal change in dipole moment between its ground and excited states.

Interestingly, methanol despite being a highly polar protic solvent capable of hydrogen bonding did not show substantial increase in fluorescence intensity compared to Dimethyl sulphoxide and Acetonitrile. This indicates that hydrogen-bonding interactions in protic solvents like methanol do not significantly affect the excited-state properties of Riociguat.

Moreover, fluorescence emission in water (the most polar solvent) was lower compared to organic solvents, possibly due to dynamic quenching by hydrogen bonding or aggregation effects.

Overall, these results suggest that the fluorescence of Riociguat is predominantly solvent-dependent, with only major variations observed, consistent with its relatively rigid, non-charge-transfer molecular nature.

Sr. No	Solvents	Fluorescence Intensity
1	Water	749.124
2	DMSO	345.540
3	DMF	749.124
4	ACN	198.422
5	MEOH	45.97
6	DCM	15.56

Table 1: Effect of solvent on fluorescence intensity

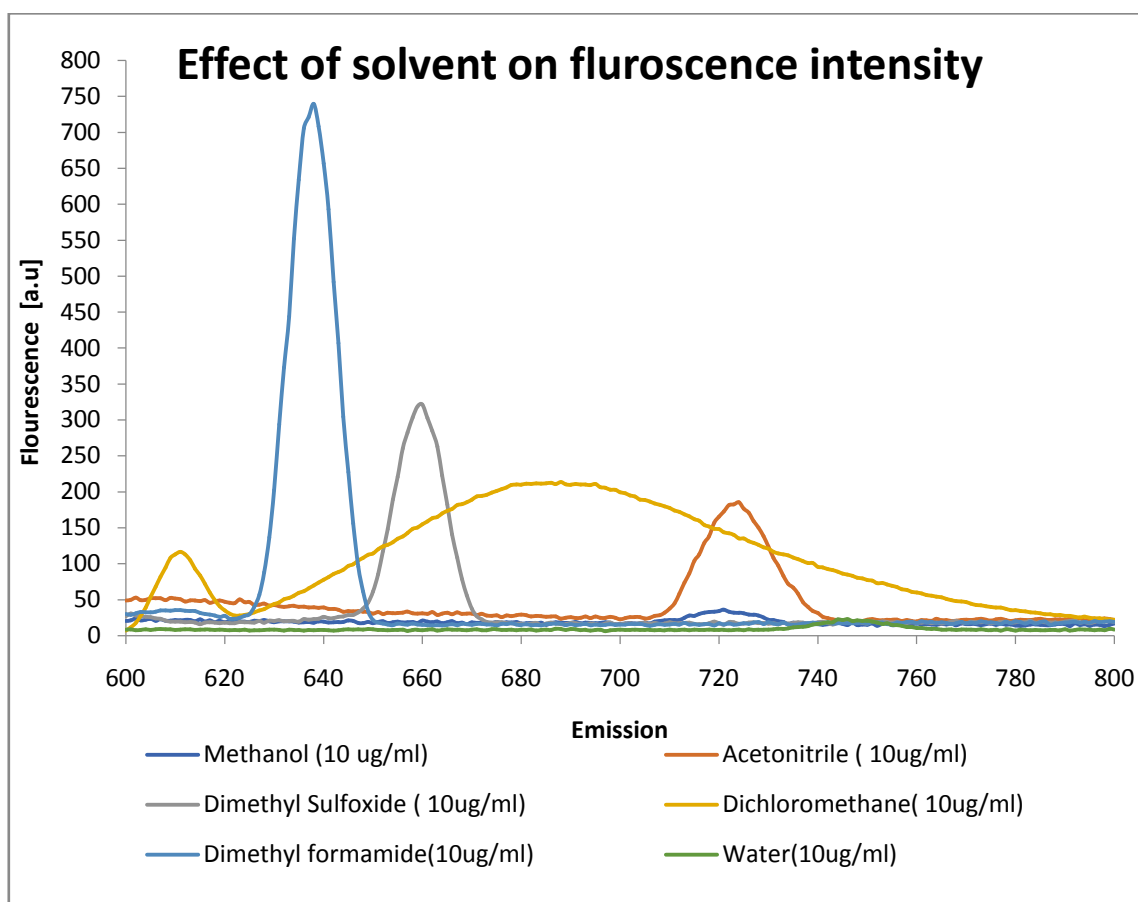


Fig 3: Depiction of effect of solvent on fluorescence intensity

#### IV. CONCLUSION

The fluorescence emission of Riociguat is strongly influenced by the nature of the solvent. Polar protic solvents like water and methanol led to reduced fluorescence intensity, likely due to hydrogen bonding and dynamic quenching. DMSO and DMF showed higher intensity, suggesting that Riociguat exhibits better fluorescence in polar aprotic environments. These results highlight the importance of solvent selection in fluorescence-based analytical methods for Riociguat.

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