Spectroflurometric estimation of droxidopa bulk and pharmaceutical dosage form

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Abstract : Simple, accurate and precise method was developed and validated for the selected drug like Droxidopa. The method is based on the spectrofluorimetric estimation of Droxidopa in drug and dosage form. Fluorescence intensity for droxidopa was measured at the excitation wavelength $^{\lambda}$ex 295 nm and emission wavelength $^{\lambda}$em 318nm. The calibration plot was obtained in the concentration range of 15 to 30 µg/mL for droxidopa. The obtained correlation coefficients are in the range of 0.999. The % recovery value are found to be 98% for droxidopa. Limit of detection and limit of quantification are found to be 1.3 and 4.0 ug/ml for droxidopa. The method was validated as per ICH guidelines and the results of validation parameters such as linearity, precision, accuracy, Limit of detection and limit of quantification indicates the suitability of the method for the routine analysis in drugs and dosage form.

Index Terms : **:** Droxidopa,Hydrochloride,HCl, spectrofluorimetry, validation.

I.INTRODUCTION :

Droxidopa is a precursor of noradrenaline that is used in t Droxidopa is a medication used to treat symptomatic neurogenic orthostatic hypotension (nOH) caused by dopamine beta-hydroxylase deficiency, non-diabetic autonomic neuropathy and primary autonomic failure caused by conditions such as Parkinson's disease.

Droxidopa is a precursor of noradrenaline that is used in the treatment of the Parkinsonism. It is approved for use in Japan and is currently in trials in the U.S. The racemic form (dl-threo-3,4-dihydroxyphenylserine) has also been used, and has been investigated in the treatment of orthostatic hypotension. There is a deficit of noradrenaline as well as of dopamine in Parkinson's disease and it has been proposed that this underlies the sudden transient freezing seen usually in advanced disease.

Droxidopa is an orally active synthetic precursor of norepinephrine that increases the deficient supply of norepinephrine in patients with NOH, thereby improving orthostatic blood pressure and alleviating associated symptoms of lightheadedness, dizziness, blurred vision, and syncope through the induction of tachycardia (increased heart rate) and hypertension.

Droxidopa crosses the blood-brain barrier where it is converted to norepinephrine via decarboxylation by L-aromatic-amino-acid decarboxylase. Norephinephrine acts at alpha-adrenergic receptors as a vasoconstrictor and at beta-adrenergic receptors as a heart stimulator and artery dilator[5.]

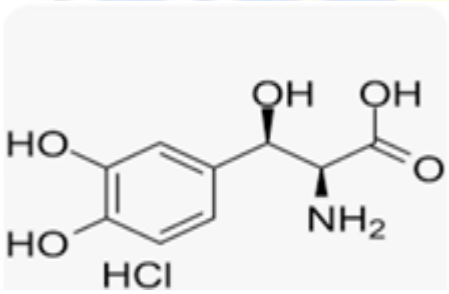

Fig.1:Chemical structure of Droxidopa

II. MATERIALS:

Instruments:

A. Digital balance Shimadzu AUX 220D model
B. Ultrasonicator
C.Melting point apparatus
D.Shimadzu FTIR 8000 series model-Spectrophotometer
E.Shimadzu RF 5301PC model-Spectrofluorometer

Chemicals and reagent:

All the chemicals and reagents used in the spectrofluorimetric analysis were of AR grade. A pharmaceutically pure sample of Droxidopa obtained as gift sample from Hetero drugs pvt ltd (Hyd,India). Hydrochloric acid,Methanol, Acetonitrile, Chloroform, Acetone, DMSO, Ethanol, Hydrochloride.

III..METHOD DEVELOPMENT:

Preparation of standard stock solution of Droxidopa:
After precisely weighing ten mg of droxidopa, and transferred to a 10 ml volumetric flask and the volume was adjusted with 0.1N HCl. The result is 1000µg/mL for the solution. Using HCl, 1 mL of the solution was collected and diluted to 10 mL in order to get 100 µg/mL. Again, 1 mL of the solution was collected and diluted with 10 mL of HCl. After precisely weighing ten milligrams of droxidopa, they were transferred to a ten-millilitre volumetric flask and the volume was adjusted with 0.1N HCl. The result is 1000µg/mL for the solution. Using HCl, 1 mL of the solution was collected and diluted to 10 mL in order to get 100 µg/mL. Again, 1 mL of the solution was collected and diluted with 10 mL of HCl.

Selection of solvent:
The fluorescence intensities of the droxidopa was measured in the solvents like methanol, ethanol, acetonitrile, acetone, DMF, DMSO and by the various pH- buffers. The Stock solution was prepared by using10 mg of the Droxidopa bulk drug and are dissolved in 10ml of solvent to prepare 1000 µg/ml of the solution. From this 0.1ml of solution is made up to 10ml in volumetric flask to prepare 10 µg/ml with the respective solvent and scanned in Spectrofluorimetry.

Selection of 0.1 N HCl[2]:
The fluorometric potential of Droxidopa was measured by dissolving various solvents (as mentioned above). The intensity was found to be good with the HCL. Hence, the HCL was further optimized for different concentrations i.e.5,10,15,20,25,30 µg/mL for Droxidopa.

calibration curve:
The solution of droxidopa (100 µg/mL) was used to prepare diluted standards. The serial dilutions of drug solutions were made by pipetting appropriate volumes of droxidopa 0.5 ,1.0 ,1.5, 2, 2.5 and 3 mL from 100 µg/mL) into the 10 mL volumetric flask and diluted up to the mark with HCL. The above dilutions gave final concentration of the 5,10,15,20,25,30 µg/mL. The fluorescence intensities of the above prepared solutions were measured at the chosen wavelength.

Assay:
Three tablets of (NORTHERA), each containing 100mg of the droxidopa was taken and accurately weighed. The average weight of the tablets was determined and crushed into fine powder. An accurately weighed quantity of powder(10 mg) equivalent to 10 mg of droxidopa was transferred to volumetric flask of 10 ml capacity. About 5 ml of 0.1N HCL was added to is volumetric flask and sonicated for 15min.The flask was shaken and volume was made up to the mark with the above solution was filtered through whatsman filter paper. From the filtrate 1 ml was transferred into 100ml volumetric flask and the volume was made upto the mark with 0.1 N HCL to give a solution containing 10**µg**/ml of droxidopa. This solution was used for the estimation of droxidopa[8]. The amount of droxidopa present in the solution was determined by substituting the responses into the equation of the straight line representing the calibration curve for droxidopa.

Method validation:
The method validated [6]according to International Council of Harmonizationguidelies for parameters like linearity, accuracy, precision, the limit of detection(LOD), and the limit of quantification(LOQ) of the analyte.

Linearity:
The solution of droxidopa (100 µg/mL) was used to prepare diluted standards. The serial dilutions of drug solutions were made by pipetting appropriate volumes of droxidopa 0.5 ,1.0 ,1.5, 2, 2.5 and 3 mL from 100 µg/mL) into the 10 mL volumetric flask and diluted up to the mark with HCL. The above dilutions gave final concentration of the 5,10,15,20,25,30 µg/mL. The fluorescence intensities of the above prepared solutions were measured at the chosen wavelength.

Accuracy (Recovery studies)[6]:
The accuracy was determined by standard addition method. Three different levels (80, 100 and 120%) of standardsof the Empagliflozin were spiked to the commercial tablets in triplicate. The mean of percentage recoveries and %RSD values was calculated and are reported in Table 4.6. The % recoveries of the Empagliflozin for three levels was found to be satisfactory.

Precision:

The repeatability (intra-day precision) of the method was determined [7]by intra-day (n=3) analysis of the three standard solutions at the concentrations of 10,15, and 20 µg/ml for droxidopa and Intermediate precision was determined[7] by the inter- day (n=3) analysis of three standard solutions of at the concentrations of 10,15and 20 µg/ml for droxidopa. In both intra- day and inter- day precision studies[5], the %RSD of repeatability was found to be within acceptance criteria (less than 2.0). Thus, the results indicated good precision of the developed method.

IV RESULTS AND DISCUSSION:

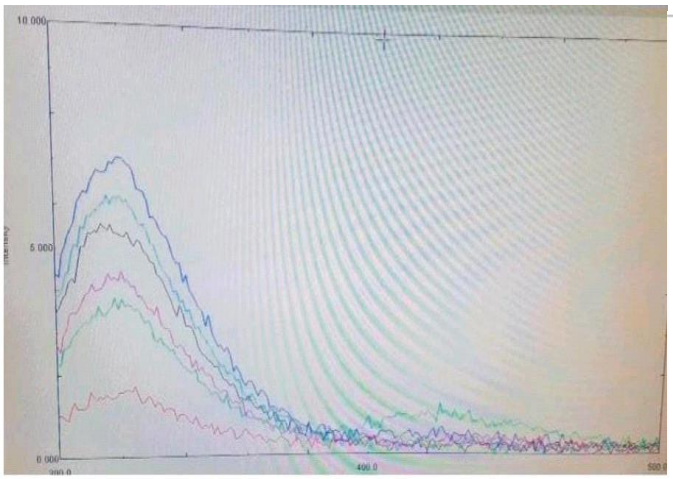

Fig: 1 Linearity of droxidopa(5-30 µg/ml)

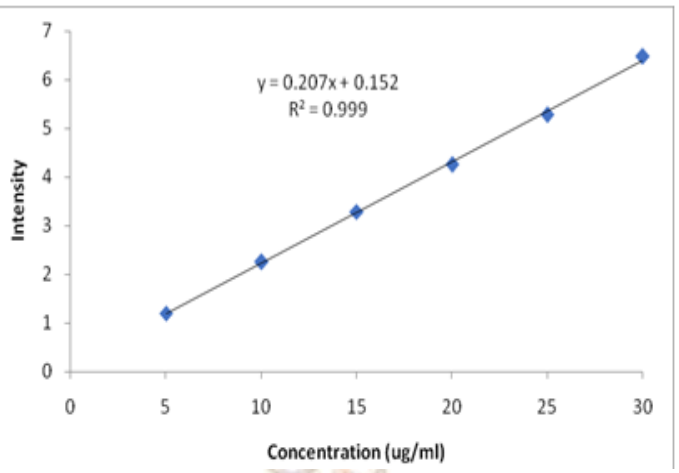

Fig: 2 Calibration of droxidopa (5-30ug/ml)

Sl.no:	Parameter	Droxidopa
1.	Excitation wavelength (nm)	295
3.	Emission wavelength (nm)	318
4.	Linearity range (µg/mL)	5 to 30
5.	Slope (m)	0.2112
6.	Intercept (c)	0.0818
7.	Regression equation	y = 0.2112x + 0.0818
8.	Correlation coefficient (R^2)	0.999
9.	Accuracy (%RSD)	0.215
10.	Precision ((%RSD)	0.101
11.	LOD (µg/mL)	1.330
12.	LOQ (µg/mL)	4.0326

Table 1:system suitability paramaeters

V.CONCLUSION :

The present work was attempted with the objective to estimate spectrofluorimetric method for the Droxidopa in Bulk and Dosage form. Droxidopa exhibited the good fluorometric intensities in the solvent 0.1N HCl[4.] Simple spectrofluorimetric method was developed for the estimation of Droxidopa at emission wavelength 318 nm with linearity in the range of R^2 value 0.999. The contemplated analytical method [1] was validated as per the ICH guidelines. The % relative standard deviation (%RSD) found to be less than 2, which denotes the immense scope of sensitivity, precision, accuracy and system suitability of the analytical method. LOD and LOQ values obtained were 1.330 µg/mL and 4.0236 µg/mL for the Droxidopa. Northera content was estimated by the contemplated analytical method in marketed tablets. The % assay of Droxidopa was found to be 98% .The assay values were in good agreement with the labeled claims, which was evidenced with % RSD value, denoting interference of excipients in the drug estimations was not found. Based on above results, we concluded that the proposed analytical method is employed routine quality control analysis of Droxidopa in tablet dosage form[8.]

VI.REFERENCES.

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