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Analytical Method Development and Validation of Droxidopa By Using HPLC Method

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ABSTRACT

A specific, accurate, precise method developed and validated for the estimation of droxidopa. By using High Performance Liquid Chromatography and chromatographic system was equipped with Inertsil column C18 column (150mm x 4.60 mm, 5µ) as stationary phase and UV detector set at 250 nm, in conjunction with a mobile phase of 0.1%v/v Triethylamine in Water 60:40% v/v ratio at a flow rate of 1.0ml/min. The described method was linear over a concentration range of 50-150 µg/ml for droxidopa. The retention time of Droxidopa is 3.33 min and %RSD less than 2.0 found for six standard replicated injections in System precision. In Method precision %Assay also found between 90-110%, the % recovery of Droxidopa were found to be 99.10% - 101.01 % respectively. Method were validated for Linearity, accuracy, precision, specificity, Robustness according to ICH guidelines and can be used for analysis. So, the method can be used for routine analysis.

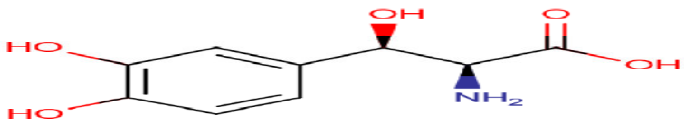
Keywords: Droxidopa, Inertsil ODS, Triethylamine and Acetonitrile

INTRODUCTION

Droxidopa https://go.drugbank.com/drugs/DB06262

Droxidopa is a precursor of noradrenaline that is used in the treatment of Parkinsonism. It is approved for use in Japan and is currently in trials in the U.S. The racaemic 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 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.



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.

MATERIALS AND METHOD

HPLC analysis carried out using HPLC system (Shimadzu(LC 20 AT VP) equipped with a PDA detector,Inertsil ODS 3V C18 column (150mm,3.20mm), Lab solution software. Analytical balance (Sartorius Balance), pH meter (Global digital), Citizen, Digital Ultrasonic Cleaner were used in the study.

Chemicals and reagents

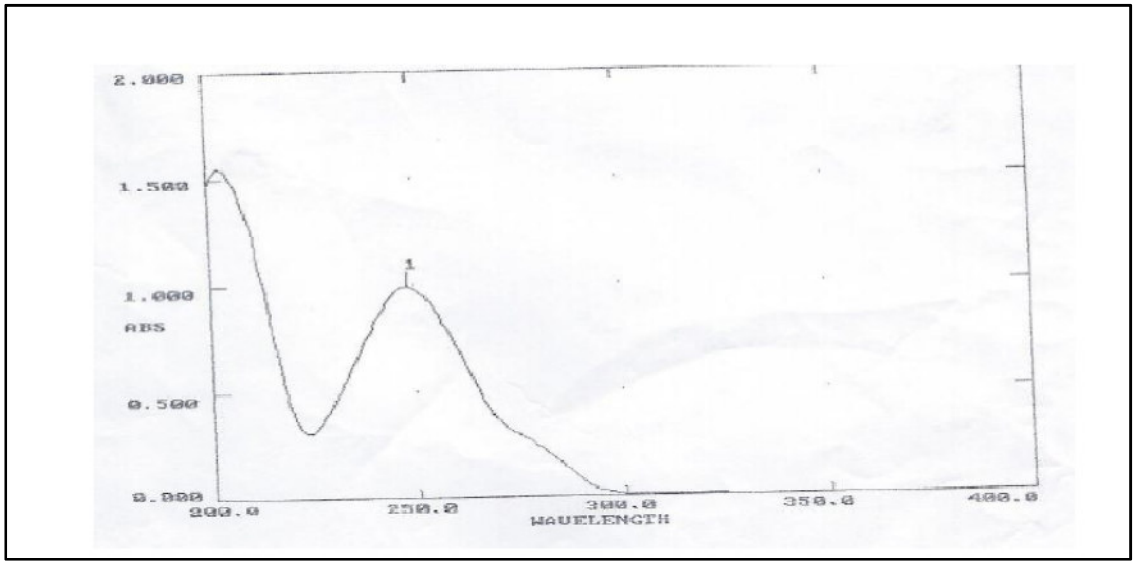
Water (Milli-Q &HPLC Grade), Methanol(HPLC Grade, Make: Merck), Triethylamine (HPLC Grade, Make: Merck), Acetonitrile(HPLC Grade, Make: Merck), Droxidopa API gifted sample from Chandralabs.

Solubility Studies

It is slightly soluble in water. It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol.

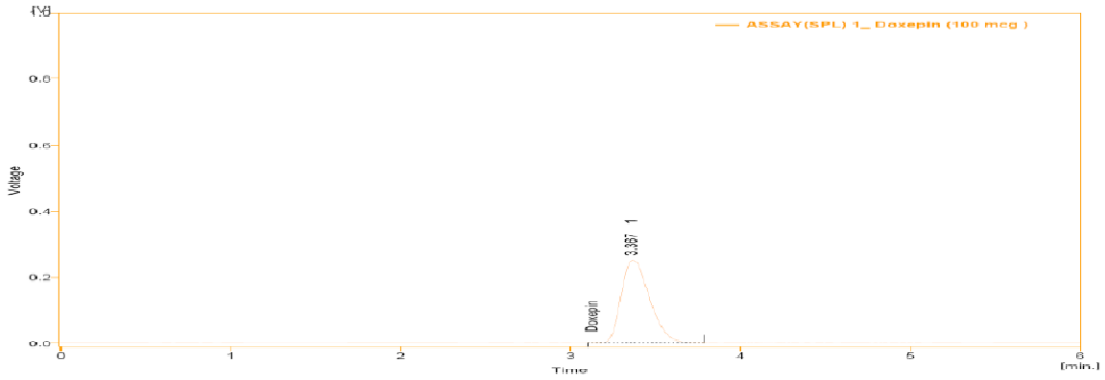
Deterimination of Maximum Absorbance by Using UV-Vi spectroscopy

The above prepared 10 µg/ml con of solution was allowed to scan amid 200-400nm to identify the wavelength using methanol as blank. The wavelength was found at 250nm.



UV-VIS spectrum of Droxidopa at 250nm

Optimised Trial



Optimized Chromatogram

Optimised conditions

Mobile phase	0.1% Triethyl amine in water :Acetonitrile (60:40)
pH	5.1
Column	Inertsil ODS 3V column,C18(150x4.6mm) 5µm
Flow rate	1.0 ml/min

Column temperature	25°C
Sample temperature	25°C
Wavelength	250nm
Injection volume	20µL
Run time	6min
Retention time	About 3.317min for Droxidopa

Preparation of Buffer

Accurately taken 1mL of Triethylamine in to 1000mL of Water and adjusted pH 5.1 with orthophosphoric acid, filtered through 0.45µ membrane filter.

Preparation of Mobile Phase

Mixed 600mL of above buffer and 400mL of Acetonitrile, degassed by sonicator.

Standard solution

Weighed 25mg of Droxidopa in 25ml of volumetric flask and dissolved in 10ml of mobile phase & made up the quantity with mobile phase From on top of stock solution 100µg/ml of Droxidopa is equipped by diluting 1ml of Droxidopa to 10ml with mobile phase.

Sample solution Preparation

5tablets (each tablet contains 250mg of Droxidopa) were weighed & make a fine power. Crushed with mortar and pestle and weighed and transferred equivalent to 100mg of Droxidopa in to 100mL VF and added 70mL of mobile phase and sonicated for 30min, final volume made upto mark and mixed well, centrifuged, further diluted 1mL to 10mL with mobile phase.

System Suitability parmeters System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to beanalyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure beingC. System suitability tests are an integral part of liquid chromatography. These tests include tests for System suitability test parameters like Retention time, Theoretical plate, Tailing factor are shown in table

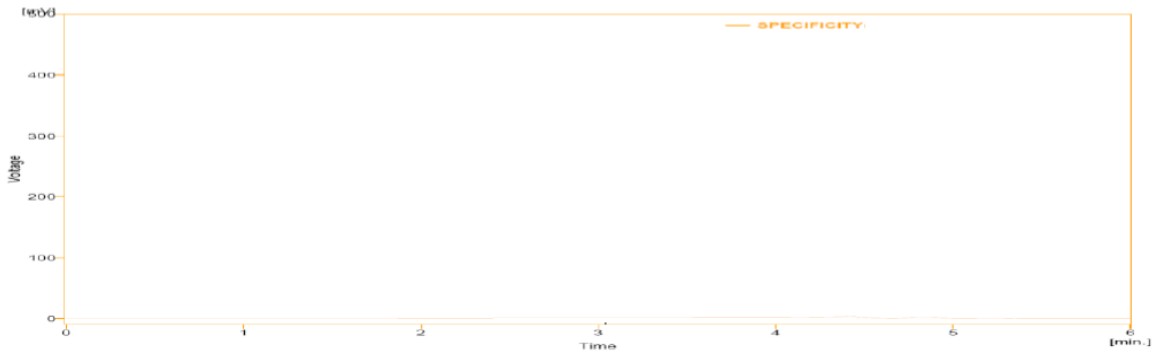
Name	Retention time	Tailing factor	Theoretical Plates
Droxidopa	3.3min	1.1	4521

Validation

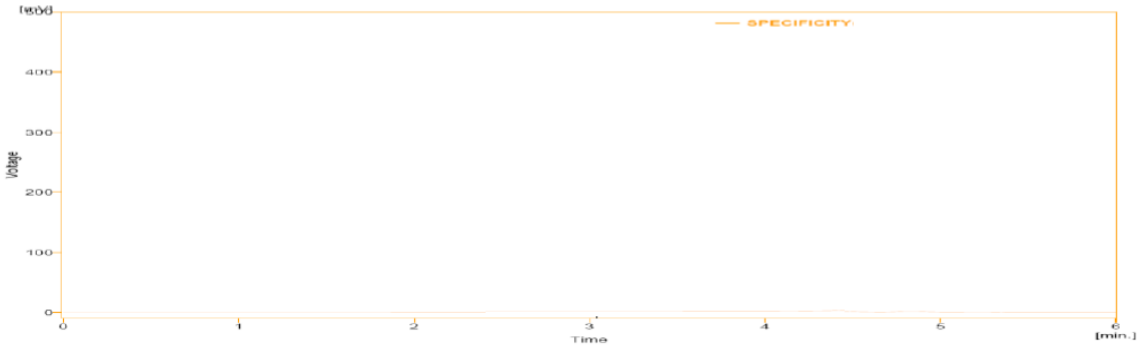
Specificity

Specificity is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix ,In case of an HPLC method, it is assured by complete separation of peak(s) of analyte(s) from other peaks originated from the sample matrix.

Blank and Placebo were not interfeared at the retention time of the main peak.



Blank Chromatograms



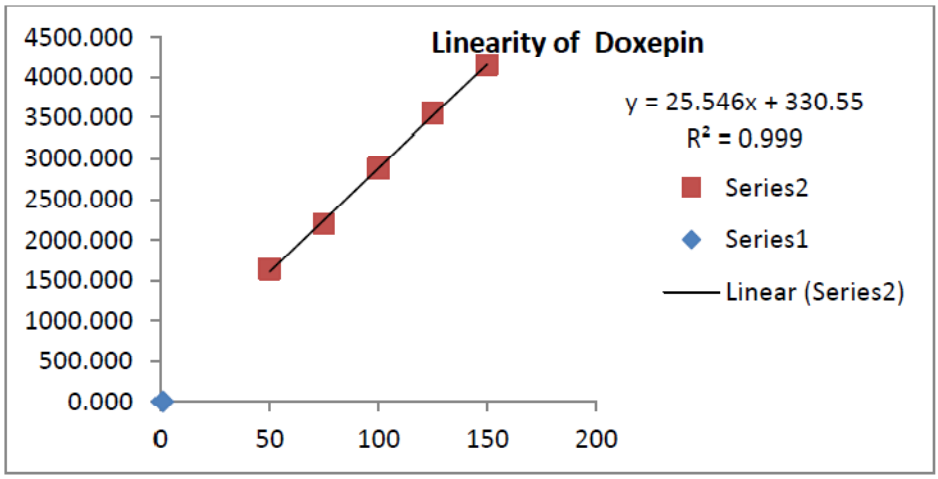
Placebo Chromatograms

Linearity

The linearity of the response for droxidopa was determined by preparing standard solutionswith concentrations of 50-150µg/ml. The calibration curves of droxidopa and shown in Figurerespectively indicate that the response is linear over the concentration range studiedwith correlation coefficient (r) value 0.999. Overlain peaks forDextromethorphan in their linearity range shown in Figure

S.No.	Conc.(µg/ml)	Area
1	50	1638.768
2	75	2197.919
3	100	2882.593
4	125	3550.790
5	150	4155.542

Linearity Table



Linearity graph

Accuracy

Accuracy was performed at 50%, 100% and 150% levels by standard addition method. Eachconcentration was analyzed 3 times and average recoveries were measured.Accuracy of the methods was assured, involving analysis of formulation samples to which certain amounts of authentic drug was added. The resulting solution was assayed, and the results obtained for drug was compared to those expected. %Recovery found within the acceptance criteria i.e., 98-102%

Recovery level	% Recovery	Average % Recovery
75%	99.58	
100%	99.10	
125%	101.01	99.89

Observation

The% recovery of Droxidopa is 99.89%.

Method Precision

Precision of the method was determined by sample solutions. Precision was determined in six replicates of

sample solution (100□μgmL). The results were expressed as %RSD and %Assay of the measurements.The resulting solution was assayed, and the results obtained for drug was compared to those expected. %Assay found within the acceptance criteria i.e., 90-105%, %RSD for six replicated preparation were found below 2.0%

Name	%Asasy
Method Precision-01	99.3
Method Precision-02	100.2
Method Precision-03	100.4
Method Precision-04	98.9
Method Precision-05	99.6
Method Precision-06	101.1
%RSD	0.78

Robustness

To determine the robustness of the current method, the effect of flow rate was studied at 0.8 and 1.2□mLmin^{-1} instead of 1.0 mLmin^{-1}. The effect of wavelength was studied at 248nm and 252nm instead of 250nm. The %RSD of robustness testing under these conditions was calculated in all cases.

Parameter	Droxidopa	
Retention time(min)	Tailing factor	
Flow	4.187	1.117
0.8ml/min	3.367	1.132
1.0 ml/min	2.830	1.156
1.2ml/min		

Wavelength	3.353	1.605
248nm	3.367	1.632
250nm	3.353	1.605
252nm		

CONCLUSION

The developed RP-HPLC method for the determination of Droxidopa is simple, precise, accurate, reproducible, and highly sensitive. The developed method was validated based on USP and ICH guidelines. Hence, this method can be used for the routine determination of Droxidoapa

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