



## Method development and validation of droxidopa by HPLC technique

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### ABSTRACT

The purpose of this work is to develop and validate stability, indicating reverse phase High-performance liquid chromatography (HPLC) method for the rapid and precise determination of droxidopa in its pure form and formulations. A simple, fast, accurate and economical way has been developed and validated for the quantification of droxidopa by HPLC technique. The chromatographic system was equipped with Shimpack column C<sub>18</sub> ((250x 4.6) mm, 5 $\mu$ ) as stationary phase and UV detector at 220 nm, in conjunction with a mobile phase of phosphate buffer pH 2.0: acetonitrile (60:40, % v/v) at a flow rate of 1.0 mL/min. The developed HPLC technique was found to be rapid as the retention time was 2.2 minutes for droxidopa peak to elute. The method was validated as per the International Conference on Harmonization (ICH) guidelines for specificity linearity, accuracy, precision. The developed method was selective with well-resolved peak. Linearity was observed over the concentration range of 25-150  $\mu$ g/mL for droxidopa. The recovery of Droxidopa was found to be 100.54% - 101.65%. Statistical techniques were employed for the validation of precision, linearity, accuracy, robustness and ruggedness and can be applied for routine analysis. Validation revealed that the developed method was specific, accurate, precise, reliable, robust, reproducible and suitable for the systematic quantitative review.



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### INTRODUCTION

Orthostatic hypotension is a significant problem which affects skeletal- motor actions, multiple sys-

tem degeneration as well as in Parkinson's disease. Treatment using pharmacological measures in maintaining a sufficient amount of the noradrenaline is not just enough because the damage to nerve terminals or ganglia or central autonomic networks occurs. A preferred way is prescribing sympathomimetics along with the other pharmacological agents that affect physiological factors which help to reduce blood pressure. Droxidopa is chemically L-threo-dihydroxyphenylserine (Figure 1), is a pro-drug, which differs from noradrenaline by a carboxyl group. It can be allowed to take orally, unlike noradrenaline (Mathias, 2008). Droxidopa is a pro-drug of noradrenaline, which escalates the amounts of neurotransmitters in the human body and brain (Goldstein, 2006).

A literature survey reveals that only one method

based on HPLC (Ankit *et al.*, 2017) is available for determination of droxidopa in the formulation and HPLC and LC-MS/MS (Chen and Hewitt, 2018; Derangula *et al.*, 2018; Wang *et al.*, 2016) methods have been reported for estimation of drug and its metabolites in biological fluids. These methods are complicated, costly, and time-consuming with complicated mobile phases and gradient elution, leading to baseline shifting in comparison to a simple HPLC-UV process. Overall the presented method was to establish specific, robust, accurate, optimised and validated RP-HPLC technique which can be applied for the quantification of droxidopa in the presence of degradants. The optimised isocratic HPLC technique was validated cohering to the ICH guidelines (ICH, 2005).

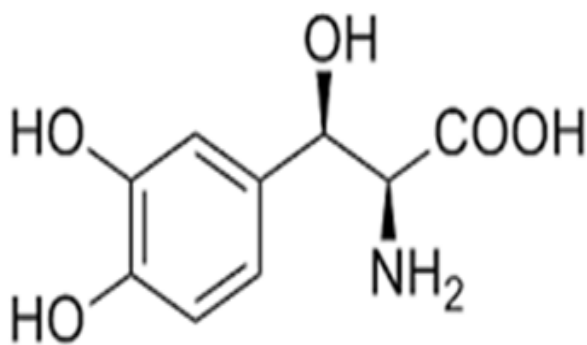


Figure 1: Chemical Structure of Droxidopa.

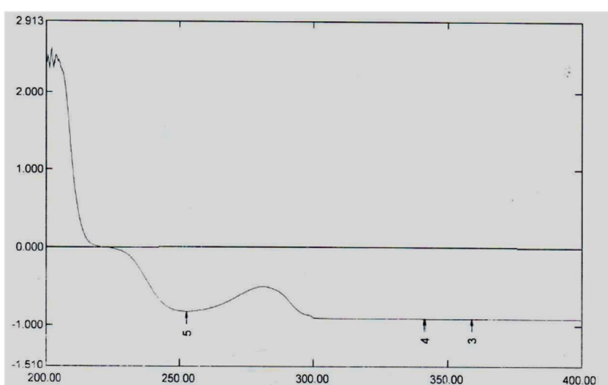


Figure 2: UV spectrum of droxidopa

## Experimental

### Chemicals and Reagents

Droxidopa was obtained as a gift sample from the reputed pharmaceutical organisation. Potassium dihydrogen phosphate, orthophosphoric acid, HPLC grade acetonitrile, water for HPLC was sourced from Rankem, India.

### Preparation of phosphate buffer

Potassium dihydrogen phosphate (0.01 mM) in 990mL of HPLC water and pH fixed at 2.0 using

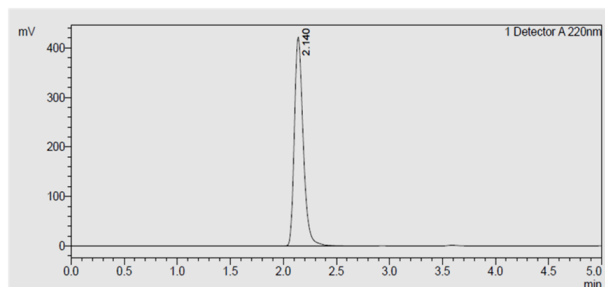


Figure 3: Typical HPLC chromatogram of droxidopa sample

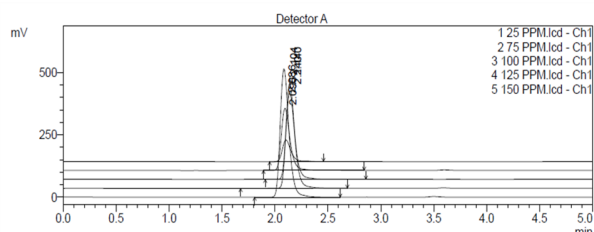


Figure 4: Linearity overlap for droxidopa

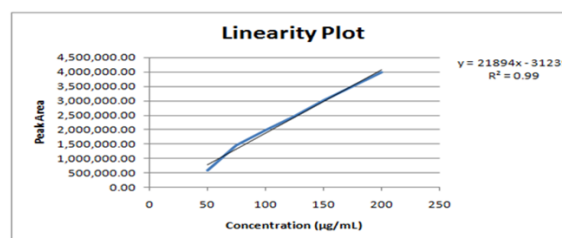


Figure 5: Linearity graph for droxidopa

orthophosphoric acid and made up to 1000mL with water, Filter the buffer solution.

### Mobile phase proportion

Phosphate buffer: Acetonitrile(60:40, %v/v)600mL of buffer and 400mL of acetonitrile.

### Liquid chromatography - UV spectrometry

The Shimadzu high-performance liquid chromatography consists of LC/10 AT/VP solvent delivery system, 7725i rheodyne injector with 20 $\mu$ L loop, SPD M/30A PDA detector and Class VP data station software. Analytical balance (Semi-micro) make Shimadzu AUW220D), Ultra-sonicator (LMUC-12) make: Spectrum Tek, Digital pH meter (PH12-5p-920) make: Spectrum Tek, Hot air oven, In lab Equipment Private Limited.

### Wavelength Selection

From the standard stock solution, 1 $\mu$ g/mL of the solution was prepared in mobile phase by diluting 1mL of the stock solution to 10 mL. After preparing, the solution was exposed to UV (Shimadzu-1700) for the scrutinisation of wavelength between 200-400nm. The  $\lambda$  max of the drug droxidopa was found

**Table 1: System Suitability studies.**

Name	Retention time	Peak area $\pm$ SD	Theoretical plates $\pm$ SD	Asymmetry $\pm$ SD
Droxidopa	2.32	1997260 $\pm$ 0.250	4557.55 $\pm$ 0.71	1.36 $\pm$ 1.58

SD: Standard deviation, n = 6.

**Table 2: Result of forced degradation study of Droxidopa.**

Degradation method	Optimized condition	% degradation of Droxidopa
Acid	1 mL 0.1N HCl for 24 hours at 80° C	13.87
Alkali	1 mL 0.1 N NaOH for 24 hours at 80° C	10.34
Peroxide	1 mL 3% H <sub>2</sub> O <sub>2</sub> for 24 hours at 80° C	6.35
Thermal	24 hours at 80° C	12.64
Sunlight	24 hours exposed to sunlight	5.24

**Table 3: Regression characteristics determined by the proposed method**

Parameters	Droxidopa
Linearity range	25-150 $\mu$ g/mL
Slope (b)	21894
Intercept (a)	31239
Correlation coefficient (r)	0.992
LOD	2.521ppm
LOQ	7.534ppm

**Table 4: Accuracy Data of Droxidopa**

Drug	Accuracy level	Amount of drug ( $\mu$ g/mL)	Quantity added ( $\mu$ g/mL)	Recovered ( $\mu$ g/mL) $\pm$ SD (n=3)	% Accuracy $\pm$ SD (n=3)
Droxidopa	Level-1	100	60	163.08 $\pm$ 0.77	101.92 $\pm$ 0.48
	Level-2	100	100	202.26 $\pm$ 1.11	101.13 $\pm$ 0.55
	Level-3	100	140	243.51 $\pm$ 0.82	101.46 $\pm$ 0.34

SD: Standard deviation, n = 3.

to be at 220nm (Figure 2).

### Chromatographic separation

Chromatographic separation was obtained with phosphate buffer: acetonitrile in the proportion of 60:40 %v/v with a Shimpack column C<sub>18</sub> (250 mm x 4.6 mm, 5 $\mu$ ) and asolvent speed of 1.0 mL/min., injection capacity of 10  $\mu$ L, ambient column temperature and detection at a wavelength of 220nm by PDA detector.

### Preparation of Droxidopa standard solution (200 $\mu$ g/mL)

Droxidopa working standard, 25 mg accurately weighed, dissolved in 5mL of 0.1N HCl and diluted with a suitable volume of mobile phase to 25 mL (1000 ppm). The standard stock solution was

diluted with a proper volume of mobile phase to get 200ppm.

### Sample solution

Droxidopa sample equivalent to about 25 mg accurately weighed and dissolved in 5ml of 0.1N hydrochloric acid, and the volume was made up to 25 mL with a mobile phase to produce a solution of 1000 ppm. The sample stock solution was diluted with a suitable volume of mobile phase to get 200ppm.

### Procedure for method validation

Validation of the proposed HPLC method was carried out as per the ICH guidelines Q2 (R1) for precision, linearity, accuracy, robustness and ruggedness (I H T Guideline, 2005).

**Table 5: Ruggedness**

Sl. No.	Analyst-1 (% Assay)	Analyst-2 (% Assay)
1	101.07	100.67
2	100.54	99.50
3	101.05	99.81
4	101.17	101.31
5	100.66	102.32
6	100.47	99.88
mean	100.83	100.58
SD	0.305	1.080
%RSD	0.30	1.07
Overall mean	100.71	
Overall SD	0.77	
Overall %RSD	0.76	

\*% RSD: Percentage relative standard deviation;  
SD: Standard deviation, n = 6.

### System suitability

To establish an HPLC testing system meets the requirement for the purpose; it was verified by injecting six replicates of standard solution and various parameters like tailing factor, theoretical plates were evaluated statistically.

### Specificity

Specificity in the method is the competence to measure the analyte in the presence of its excipients and degradation products. Commonly used formulation excipients like cellulose derivatives, starch, sucrose-based polyvinyl pyrrolidone, polyethylene glycol and lactose were spiked into a pre-weighed quantity of droxidopa; appropriate dilutions made and tested how well the method can recognise the analyte.

### Forced Degradation Studies

Forced Degradation Studies of droxidopa, was performed at various stress conditions as specified in ICH guideline Q1A (R2) (ICH, 2005). The droxidopa solution containing 100 µg/ml was exposed to the acidic, alkaline, oxidative, thermal condition. Acidic and alkaline degradation was performed at 80°C temperature and neutralised before injecting. Oxidative stress studies were carried out for using 3% H<sub>2</sub>O<sub>2</sub> (Reynolds *et al.*, 2002).

The degraded samples, along with droxidopa control sample, were chromatographed and examined for any interferences at the retention time of the droxidopa peak from the potential degradants.

### Precision

The precision was assessed by performing repeatability at a target drug concentration of 100 µg/mL in

one day, and % RSD was calculated. The precision studies were also repeated on subsequent days to determine intermediate precision and by different analysts.

### Linearity and range

Five levels of calibration solutions at a concentration from 25 to 150 µg/mL were prepared and diluted with the mobile phase from the standard stock solution.

Calibration curve designed by plotting peak area against concentrations of droxidopa, correlation coefficient calculated.

### Limit of detection and limit of quantification

LOD is the smallest concentration of the analyte which involves an estimable response (Signal to noise ratio 3) whereas LOQ is the level at which precision is weaker than a specific value (RSD ≥ 3.0% or Signal to noise ratio 10)

$$1. \text{ LOD} = 3.3 \times \sigma / S$$

$$2. \text{ LOQ} = 10 \times \sigma / S$$

Where  $\sigma$  is the standard deviation of y-intercepts of regression lines, and S is the slope of the calibration curve.

### Accuracy

Method accuracy confirmed by recovery experiments. The accuracy was assessed by triplicate determinations of three different solutions having 160, 200 and 240 µg/mL.

## RESULTS AND DISCUSSION

### HPLC method development and optimisation

To attain the best separation conditions, the optimisation of the mobile phase was to provide desirable selectivity and sensitivity in low run time. Columns from different brands were evaluated. Eventually, a symmetrical peak of the analyte with a justifiable low run time was fulfilled by engaging buffer and acetonitrile, and Shimpack column C<sub>18</sub> (250 mm x 4.6 mm, 5 $\mu$ ) was finalised, as it furnished better peak symmetry about 1.25, theoretical plates and firm baseline. The validation variables are linearity, precision accuracy, robustness for the finalised chromatographic parameters of the HPLC method of droxidopain solid dosage form and quantification for bulk and dosage forms.

### Method validation

#### System suitability

The peak symmetry and theoretical plates were calculated for the working solutions. The acceptance criteria for peak area counts should be not more than 2.0 % RSD and for tailing factor not more than 2.0 for the analyte peak. The acceptance criterion for theoretical plates was not less than 4000. The system suitability parameters finalised for the analysis of droxidopa are in Table 1.

The specificity trial displayed that pharmaceutical additives and degradation products did not intrude with the peak of the droxidopa. None of the peaks was eluted at the retention time of droxidopa (Figure 3).

This proves that the proposed method was particular for quantitation of droxidopa in the formulation (Shabir, 2003). The results of the degradation studies using different optimised condition are listed in Table 2.

#### Linearity

Acceptable linearity range was illustrated in the present work for droxidopa over the range of 50–150  $\mu$ g/mL (Jain *et al.*, 2009). A calibration curve was plotted for five standard concentrations of droxidopa versus droxidopa peak area, a regression equation was computed, and the linearity of the calibration curve (Figure 4) and linearity overlap (Figure 5) was established by the high value of correlation coefficient,  $r^2$ : 0.999 justifies the outstanding correlation linking the concentrations and peak area of droxidopa and are briefed in Table 3.

The detection limit and quantification limit were obtained from the slope of the linear regression curve. The limit of detection is 2.521  $\mu$ g/mL, and the quantification limit is found to be 7.534  $\mu$ g/mL.

### Accuracy studies

Accuracy was accomplished at three levels (80%, 100% and 120%). Triplicate analyses carried out at 160, 200 and 240  $\mu$ g/mL and average recoveries were studied and shown in Table 4.

### Method precision and Intermediate precision

The precision of the method was carried by performing six independent determinations. The average assay of six determinations of droxidopa was 100.83 with RSD of 0.303. % RSD < 2.0 indicates that the method is precise (Sabir *et al.*, 2013). The ruggedness was verified by performing the chromatographic analyses of samples by various analysts on two different days. Results are presented in Table 5.

### Robustness

There were nonnoticeable changes in the chromatographic pattern when deliberate changes were made in the chromatographic conditions, thus making the method robust.

## CONCLUSIONS

A fast, sensitive, precise, rugged, accurate RP-HPLC technique was progressed for droxidopa, and the evolved method was validated as per ICH Q2 (R1A) guidelines. The % RSD of < 2.50 % shows the HPLC technique to be precise. Excellent linearity of droxidopa is shown from 25 to 150  $\mu$ g/ml. Recovery experiments that were accomplished exhibited the accuracy of the method. This ranged between 100.54% - 101.65%. Thus it can be accomplished that an accurate, precise and rugged RP-HPLC technique has been optimised and validated for the regular estimation of droxidopa in pure and oral dosage formulations.

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### Conflict of interest

The authors declare that they have no conflict of interest for this study.

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## REFERENCES

Ankit, B., Chaudhary, R. D., Patel, M. J., Hingu 2017. Analytical method development and validation of

- droxidopa. *WJPPS*, 6(10):921–932.
- Chen, J. J., Hewitt, L. A. 2018. Comparison of the Pharmacokinetics of Droxidopa After Dosing in the Fed Versus Fasted State and with 3-Times-Daily Dosing in Healthy Elderly Subjects. *Drugs in R&D*, 18(1):77–86.
- Derangula, V. R., Thumma, J., Ponneri, V. 2018. Development and validation of LC-MS/MS method for the estimation of Droxidopa in Human Plasma. *Int Journal Chem Tech*, 11(11):232–241.
- Goldstein, D. S. 2006. L-dihydroxyphenylserine (L-DOPS): a norepinephrine prodrug. *Cardiovascular drug reviews*, 24(3-4):189–203.
- I H T Guideline 2005. Validation of analytical procedures: text and methodology Q2 (R1). 11. International conference on harmonization.
- ICH 2005. Q2 (R1): Validation of analytical procedures: text and methodology. International Conference on Harmonization.
- Jain, K. S., et al. 2009. Stability Indicating HPLC Method for Simultaneous Determination of Mephenesin and Diclofenac Diethylamine. *Indian J. Pharm. Sci*, 71(1):35.
- Mathias, C. J. 2008. L-dihydroxyphenylserine (Droxidopa) in the treatment of orthostatic hypotension. *Clinical Autonomic Research*, 18(S1):25–29.
- Reynolds, D. W., Facchine, K. L., Mullaney, J. F., Alsante, K. M., Hatajik, T. D., Motto, M. G. 2002. Conducting forced degradation studies. *Pharmaceutical technology*, pages 48–56.
- Sabir, A. M., Moloy, M., Bhasin, P. S. 2013. HPLC method development and validation: A review. *Int. Res. J. Pharm*, 4(4):39–46.
- Shabir, G. A. 2003. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of chromatography A*, 987(1-2):57–66.
- Wang, H., Yang, G., Zhou, J., Pei, J., Zhang, Q., Song, X., Sun, Z. 2016. Development and validation of a UPLC-MS/MS method for quantitation of droxidopa in human plasma: Application to a pharmacokinetic study. *Journal of Chromatography B*, 1027:234–238.