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# A SIMPLE STABILITY INDICATING ASSAY METHOD FOR ESTIMATION OF TRAPIDIL USING RP-HPLC

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## ABSTRCT

A simple validated stability indicating reverse phase high performance liquid chromatographic method has been developed for the determination of Trapidil. An isocratic separation was carried out using Princeton SPHR-100 $C_{18}$ (250 x 4.6mm, 5µ) column using mobile phase comprises of Acetonitrile: Phosphate buffer pH 3.0 in ratio 50: 50 at flow rate 1mL/min using 221nm as detection wavelength. The Retention time for drug was found to be 4.178 ± 0.050min. The linearity was found to be in the concentration ranging 10-70µg/mL. The percent mean estimation of drug was nearly equal to 100%. The developed method was found to be more selective and rapid with respective shorter time. The force degradation studies were conducted and % of undegraded drug was calculated under various stress conditions via solution and solid state analysis. The stability study indicated degradation (not showing any additional peak) upon certain extend. The proposed new, simple validated stability indicating assay method was found suitable for routine analysis of Trapidil in dosage form.

**KEYWORDS:** Trapidil, RP-HPLC, Stability indicating method, Stress degradation study.

## INTRODUCTION

Trapidil is a platelet-derived growth factor antagonist, was originally developed as a vasodilator and anti-platelet agent and has been used to treat patients with ischemic coronary heart; liver and kidney disease.

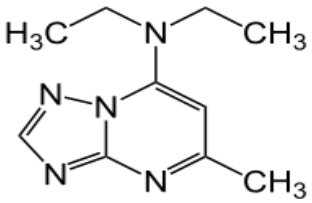

**Fig 1: Structure of Trapidil.**

Literature survey indicated that the drug has been estimated from bulk, marketed formulation and human serum by using instrumental methods like UV-spectroscopy[5], RP-HPLC[6,7] and HPTLC.[6] But no report was found to indicate stability testing of Trapidil. This paper describes simple, precise, accurate and sensitive RP-HPLC method development, validation and stability studies (hydrolysis, oxidation, photo-degradation and thermal degradation) as per international conference of harmonization guidelines.

## MATERIALS AND METHODS

### Reagents and chemicals

Trapidil was procured as gift sample from Ajanta pharmaceuticals, Mumbai. Reagents include Methanol and Acetonitrile of HPLC grade, Potassium dihydrogen phosphate, ortho phosphoric acid, Hydrogen chloride and Hydrogen peroxide of GR grade were used. Also Hydroxypropyl methylcellulose-K15, Methylcellulose, Microcrystalline cellulose, Talc and Magnesium stearate of GR grade was used for formulation.

### Instrumentation and Chromatographic condition

The HPLC system comprised Shimadzu HPLC 1100 series, PrincetonSPHR-100 $C_{18}$ column (250 x 4.6mm,5µ) and ACN: Phosphate buffer pH 3.0 (50:50 v/v) as mobile phase with quantification carried out at wavelength of 221 nm and using flow rate of 1 mL/min.

### Preparation of Standard stock solution

Standard stock solution of Trapidil was prepared by dissolving about 10mg of drug in 50mL of volumetric flask and volume was made upto the mark with methanol to get concentration of 200µg/mL.

### Working Standard solution

A 0.5mL of standard stock solution was further transferred in 10.0mL volumetric flask and volume was made upto the mark with methanol to get concentration of 10µg/mL.

**Preparation of Mobile phase**

A 7.0g of potassium dihydrogen phosphate added in 1000.0mL of double distill water and pH is adjusted to 3.0 using ortho phosphoric acid. The mobile phase comprises of Phosphate buffer pH 3.0 and Acetonitrile in the ratio 50:50. The mobile phase was mixed and filtered through 0.45µm membrane filter paper and degassed.

**Stress degradation studies**

Stress degradation studies were carried out via solution and solid state analysis.

**General procedure for Solution state analysis**

10mg of standard drug and sample equivalent to 10mg was transfered in 50mL of volumetric flask separately. To each flask 10mL of 0.1N NaOH/HCl/3%$H_2O_2$/$H_2O$ was added and kept at room temperature, $60^0$C and $80^0$C for a period of 180min. After 180min neutralization was carried out of acidic and alkaline stress sample. The content of the each flask was sonicated for 15min, volume was made upto the mark with methanol and filtered. The 2mL of these stress solution was further diluted to 10mL with methanol (Conc.40µg/mL).

**General procedure for Solid state analysis**

Standard Trapidil and sample were spread on petridish separately and kept in the oven at $60^0$C, humidity chamber ($40^0$C, 75%) and in sunlight. After 48 hours, accurately measured quantity of Trapidil standard 10mg and sample equivalent to 10mg were withdrawn and transferred to series of 50mL volumetric flask and volume was made upto the mark with methanol. The content was sonicated for 15min and filtered. 2mL of these stress samples was further diluted to 10ml with diluents (40µg/mL).

## RESULTS AND DISCUSSION

**Selection of Detection Wavelength**

From the standard stock solution further working standard solution of 10µg/mL was prepared using methanol and scanned the over the range of 200-400 nm using UV spectrophotometer. The recorded spectrum of Trapidil showed considerable absorbance at 221 nm selected as detection wavelength for development of proposed method (Fig.2).

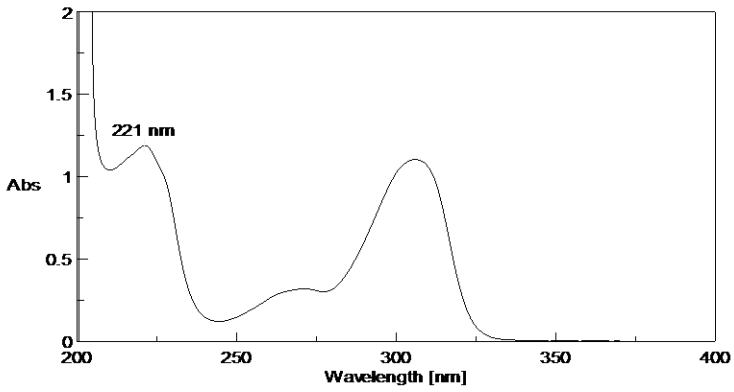


**Fig.2: UV Spectra of Trapidil.**

**Optimization of chromatographic condition**

After several trials for selection of stationary and mobile phase, stationary phase $C_{18}$ and mobile phase comprised of Acetonitrile: Potassium dihydrogen phosphate in the ratio 50:50 v/v shows sharp and symmetrical peak. The tailing factor obtained was less than two and retention time was about 4.178 min which would reduce the total run time and ultimately increase the productivity thus reducing the cost of analysis per sample.

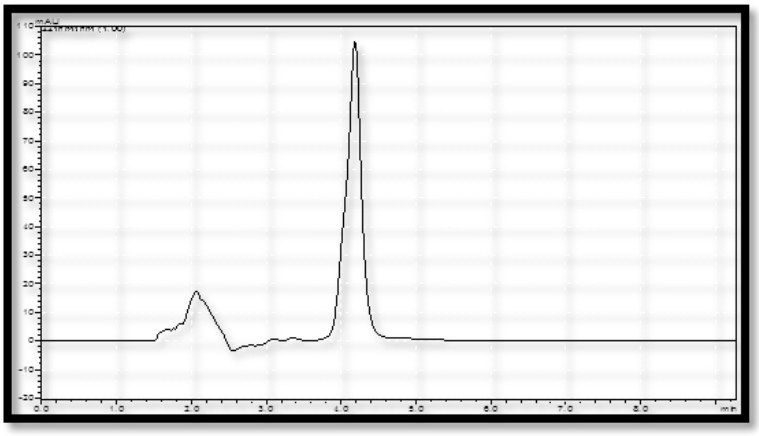

**Fig.3: Chromatogram of standard Trapidil.**

**Method Validation**

**System suitability**

System suitability was verified by replicate analysis of five injections of Trapidil working standard solution and the chromatogram was recorded. The system suitability parameters such as asymmetry, theoretical plate count and reproducibility (% RSD) of analyte retention time (Rt) and area of the five replicates were calculated from the observed chromatogram. The results indicated that optimized chromatographic conditions was found to be suitable and used for further experimentation.

**Table 1: Results of system suitability parameters.**

| Injection | AUC (mV) |
|---|---|
| 1 | 205.754 |
| 2 | 206.726 |
| 3 | 203.946 |
| 4 | 201.701 |
| 5 | 206.479 |
| **Mean** | **204.920** |
| **% RSD** | **1.03** |
| **Theoretical plate** | **13458** |
| **Retention time** | **4.178** |
| **Asymmetry** | **0.952** |

**Linearity**

From the stock solution (200µg/mL) of Trapidil, further dilutions were made with methanol to get sample solutions of seven different concentrations. Each solution injected separately and chromatogram was recorded. The linearity was determined by plotting graph between peak area and concentration over the concentration range of 10-70µg/mL of Trapidil (Fig.4). The correlation coefficient was found to be 0.997.

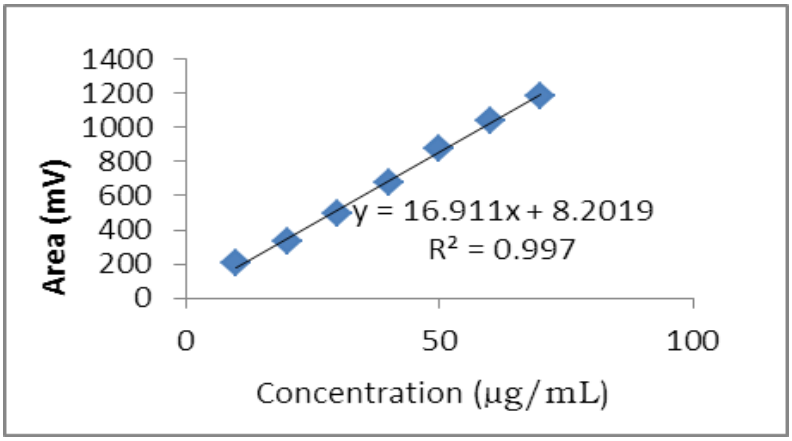


**Fig.4: Calibration curve of Trapidil.**

**Intermediate Precision**

The intermediate precision of the proposed method was demonstrated by Intraday and Intraday variation studies. In intraday studies, sample was analyzed for $0^{th}$, $3^{rd}$, $5^{th}$ hour and percentage RSD was calculated. For the interday variation studies, sample was analyzed on 3 consecutive days and percentage RSD was calculated. For intraday and interday precision %RSD was found to be 0.95 and 0.88 respectively.

**Table 2: Observation and results of intermediate presicion.**

| Intraday | | | | | Interday | | |
|---|---|---|---|---|---|---|---|
| **Sr. No.** | **Time (Hr)** | **Wt. of sample taken (mg)** | **AUC (mV)** | **% Labelled claim** | **Day** | **AUC (mV)** | **% Labelled claim** |
| 1. | $0^{th}$ | 19.41 | 332.192 | 98.31 | $1^{st}$ | 339.028 | 100.35 |
| 2. | $3^{rd}$ | | 336.483 | 99.56 | $2^{nd}$ | 338.851 | 100.25 |
| 3. | $5^{th}$ | | 338.694 | 100.15 | $3^{rd}$ | 333.927 | 98.79 |
| | | | **Mean** | **99.34** | | | **99.79** |
| | | | **±SD** | **0.939** | | | **0.873** |
| | | | **%RSD** | **0.95** | | | **0.88** |

**Precision**

Accurately weighed quantity of laboratory mixture equivalent to 10.0mg of Trapidil was transfer to 50.0mL of volumetric flask, sonicated for 15min with sufficient quantity of diluents (methanol) and volume was made up to mark with methanol. The content of flask was filtered through 0.45µm filter paper. A 1.0mL portion of the filtered was further diluted to 10.0mL with diluents (20µg/mL). After equilibration of stationary phase, five such sample solutions were injected separately and chromatogram were recorded. The content of Trapidil in each sample was calculated by comparing the peak area of sample with the Std. using formula. The mean % assay of trapidil was 101.40 indicates proposed method is presice.

**Table 3: Results for estimation of Trapidil.**

| **Sr. No.** | **Wt. of Std. taken (mg)** | **Wt. of sample taken (mg)** | **Peak area of Std. (mV)** | **Peak area of Sample (mV)** | **Amt. of drug estimated (mg)** | **% Labelled claim** |
|---|---|---|---|---|---|---|
| 1. | 10.20 | 19.21 | 329.154 | 331.253 | 10.06 | 99.55 |
| 2. | | | | 338.611 | 10.28 | 101.97 |
| 3. | | | | 339.503 | 10.31 | 102.27 |
| 4. | | | | 336.684 | 10.22 | 101.38 |
| 5. | | | | 338.245 | 10.27 | 101.87 |
| | | | | | **Mean** | **101.40** |
| | | | | | **±S.D.** | **1.086** |
| | | | | | **%RSD** | **1.07** |

**Linearity and range**

Linearity and range was performed by weighing the sample at 80-120% of label claim and dilution was made with methanol. Each solution was injected separately and chromatographed. A plot of % label claim Vs area under curve (Fig.5) indicating linearity with 0.998 as coefficient correlation value.

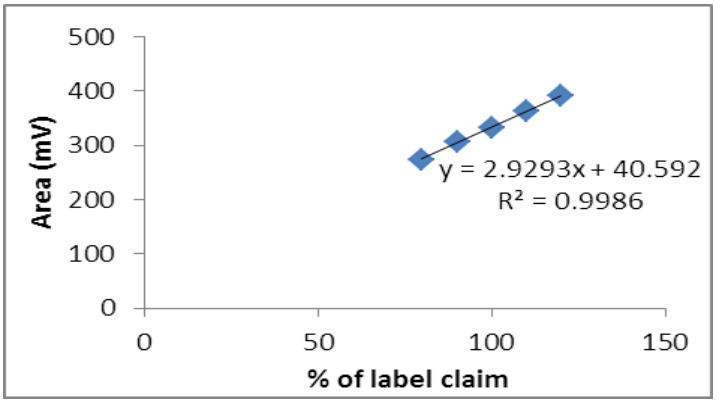


**Fig.5: Linearity curve of Trapidil.**

**Accuracy (recovery study)**

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150%. The recovery samples were prepared in the aforementioned procedure; three samples were prepared for each recovery level and analyzed using proposed method to calculate % recovery. The average recoveries of three levels for Trapidil were found to be 101.64% with 0.91 as %RSD.

**Table 4: Results of recovery study.**

| Sr. No. | Amt. of pure drug added (mg) | Amt. recovered (mg) | % Recovery |
|---|---|---|---|
| 1. | 5.01 | 5.02 | 100.60 |
| 2. | 10.20 | 10.19 | 102.39 |
| 3. | 15.30 | 15.35 | 101.95 |
| **Mean** | | | 101.64 |
| **± SD** | | | 0.932 |
| **%RSD** | | | 0.91 |

**Robustness**

The robustness of the method was evaluated by injecting the sample at deliberately varied chromatographic conditions viz. change in composition of mobile phase (±5%), wavelength (±5nm), pH(0.2) and flow rate (±0.2mL/min)and the effect on the area was noted. The method is robust as mean % RSD was found to be 1.48.

**Ruggedness**

The ruggedness was performed by using two different analysts and %RSD was found to be 1.01 and 0.81.

**Results of stress degradation study**

The result obtained by hydrolysis and oxidative study are compared with the standard drug to above mention conditions. This study explained that the rate of degradation of drug was more as the temperature get increased. Around 40-50% degradation occurs at $80^0$C as compared to room temperature. No additional peak was found under all stress conditions. It was observed that excipients have no effect on the degradation of drug in laboratory mixture. At the same time drug and its laboratory mixture was considerably stable in specified stress conditions.

**Table 5: Observation and results of solution state analysis.**

| Condition | Amt. of drug estimated | | % Estimation | |
|---|---|---|---|---|
| | Std. | Sample | Std. | Sample |
| **Alkaline Hydrolysis** | | | | |
| At room temperature | 9.63 | 9.3 | 93.83 | 90.61 |
| At $80^0$C | 5.69 | 5.17 | 55.49 | 50.37 |
| **Acid Hydrolysis** | | | | |
| At room temperature | 8.92 | 9.89 | 86.91 | 96.35 |
| At $80^0$C | 6.02 | 6.15 | 58.64 | 59.92 |
| **Neutral Hydrolysis** | | | | |
| At room temperature | 9.99 | 9.71 | 97.33 | 94.58 |
| At $80^0$C | 5.66 | 5.77 | 55.12 | 56.20 |
| **Oxidative Study** | | | | |
| At room temperature | 9.22 | 9.15 | 89.82 | 89.15 |
| At $80^0$C | 5.91 | 5.60 | 57.56 | 54.56 |

**Table 6: Observations and results of solid state analysis.**

| Condition (2 days) | Amt. of drug estimated (mg) | | % Estimation | |
|---|---|---|---|---|
| | Std. | Sample | Std. | Sample |
| Thermal study ($60^0$C) | 10.2 | 8.79 | 99.38 | 85.64 |
| Humidity study ($40^0$C, 75%) | 9.95 | 8.92 | 96.94 | 86.89 |
| Photochemical study (sunlight) | 9.66 | 9.58 | 94.10 | 93.33 |

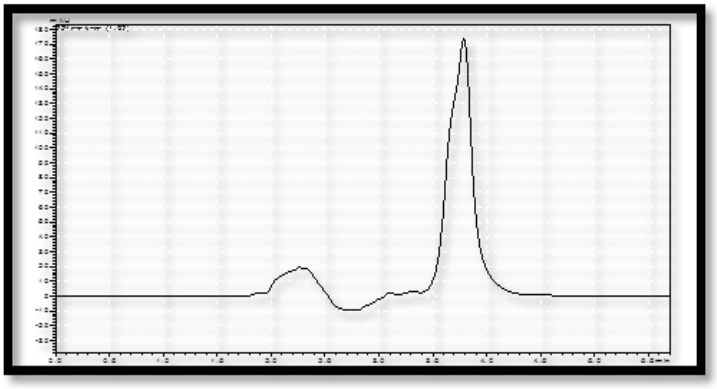

**Fig.6 (a): Chromatogram of sample at $80^0$C in 0.1N NaOH.**

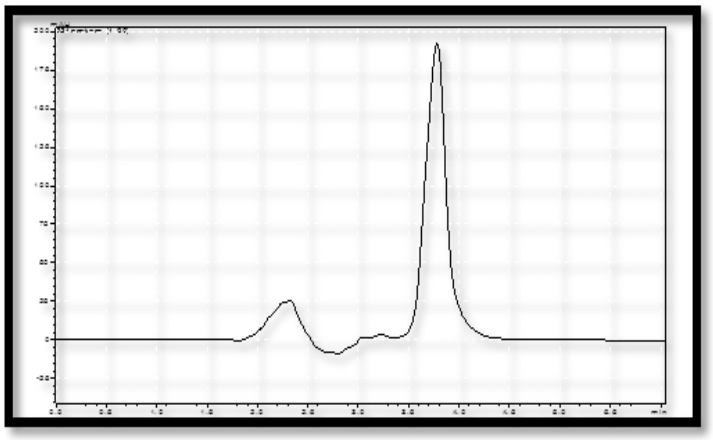

**Fig.6 (b): Chromatogram of sample at $80^0$C in 0.1N HCl.**

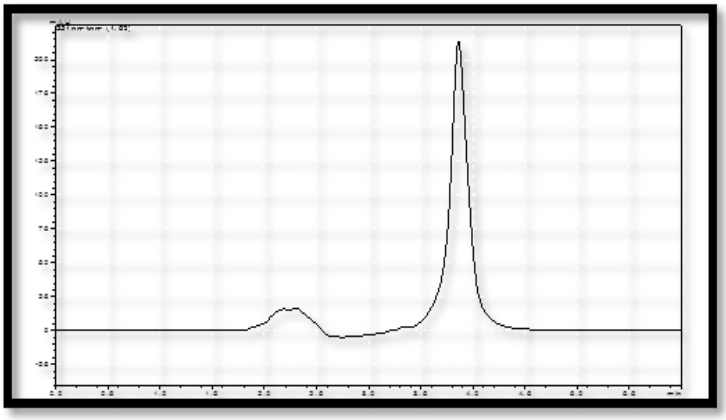

**Fig.6(c): Chromatogram of sample at $80^0$C in $H_2O$.**

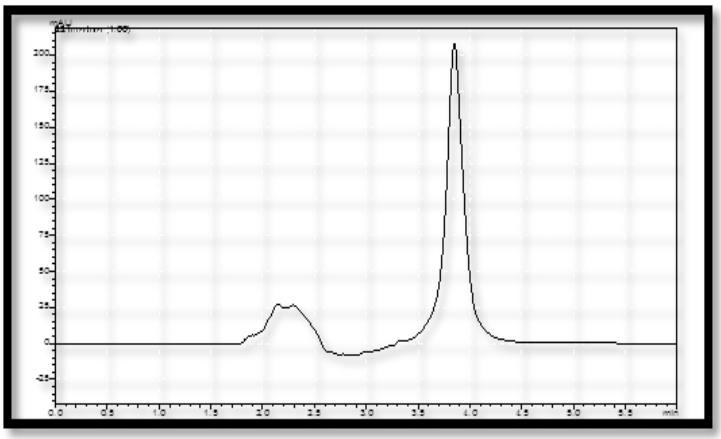

**Fig.6 (d): Chromatogram of sample at $80^0$C in 3%$H_2O_2$**

## CONCLUSION

The proposed validated RP-HPLC method is sensitive, accurate, precise and robust. Moreover, the developed method was found to be more selective and rapid with respect to shorter runtime. The stability study was performed by exposing the drug at various stress

conditions. It shows degradation (not showing any additional peak) upon certain extend. Hence, developed validated stability indicating method can be employed for the routine quality control analysis of Trapidil in laboratory mixture.

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