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Development and Validation of an RP-HPLC Method for the Estimation of Trapidil in Raw Materials and Tablet Dosage Forms.

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ABSTRACT

A simple, rapid, accurate, reliable, and sensitive high performance liquid chromatography method for the determination of Trapidil in raw materials and tablet dosage forms was developed and validated. Trapidil was chromatogramed on Hypersil Symmetry C_{18}, (100 X 4.6 mm; 5 µm) using a mobile phase consisting of phosphate buffer (pH 3.0) and acetonitrile in the ratio of 45:55 v/v. The flow rate was maintained at 0.9 ml/min and eluents were detected with the retention time of 2.22 min. at 221 nm by using UV detector absorbance. The proposed method was validated by determining accuracy, precision, and linearity was observed in the range of 10 – 50 µg/mL of the drug. The mean recovery of the drug was indicating high level of accuracy of the method. Due to its simplicity, accuracy and high precision of the proposed HPLC method was found to be appropriate for the estimation of Trapidil in bulk and pharmaceutical dosage forms.

Keywords- Trapidil, HPLC, and Validation.

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INTRODUCTION

Trapidil is chemically described as *N,N*-diethyl-5-methyl-[1,2,4] triazolo [1,5-*a*] pyrimidin-7-amine. Trapidil[1] was initially developed in 1971 as a vasodilator and anti- anginal agent and later also used as antiplatelet therapy. However, over the past few decades, studies have found Trapidil to be a potent inhibitor of platelet aggregation and activation, vascular smooth muscle cell proliferation, and monocyte/macrophage migration and activation, thereby making it a potentially potent anti- restenotic agent.

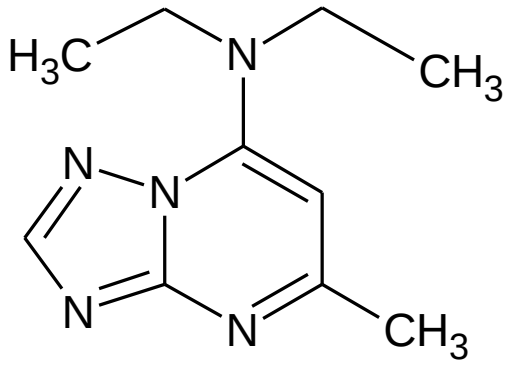

Figure 1: Chemical structure of Trapidil.

The literature survey reveals that, Trapidil was estimated by HPLC methods for its metabolites study, pharmacokinetics study in human serum and urine [4 - 7] and only one method was validated by using UV spectrophotometry, HPTLC, and RPHPLC [2]. The present method describes the rapid, accurate and precise RPHPLC method for the estimation of Trapidil in raw materials and tablet dosage forms by using different method.

MATERIALS AND METHODS

The reagents and chemicals used in this procedure are Acetonitrile, Potassium dihydrogen phosphate, ortho phosphoric acid and water of HPLC grade. A Waters 2695 model chromatograph equipped with a reverse phase Symmetry C_{18} column (100 x 4.6 mm; 5 µm) Hypersil was employed for the study.

Sample injection was performed with an automatic injector. Detection was done by a Waters 221 nm dual λ absorbance UV detector and the output signal was monitored and integrated using Waters empower software. Solubility of compound was enhanced by sonication on ultrasonic cleaner. A UV spectrum of tolterodine was taken on a UV 3000 UV visible – spectrophotometer (Labindia) in order to select the working wavelength for detection of drug. All the weights in the experiments were taken with an Sartorius electronic balance.

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Preparation of Phosphate buffer pH 3.0:

Phosphate buffer was prepared by dissolving 7.0 gm of potassium dihydrogen phosphate in 1000 mL of HPLC water. The pH of the solution was adjusted to 3.0 with ortho phosphoric acid.

Preparation of mobile phase*:*

For preparation of the mobile phase, 450 mL of phosphate buffer was mixed with 550 mL of acetonitrile, degassed in an ultrasonic bath for 5 min and filtered through a 0.45 µ membrane filter before use. The mobile phase was also used as a diluent for preparing the drug solutions. The flow rate of the mobile phase was maintained at 0.9 mL / min. The detection of the drug in the eluate was done at 221 nm.

Preparation of standard drug solutions:

About 100 mg of trapidil was weighed accurately, transferred into a 100 mL volumetric flask and dissolved in 25 mL of the diluent. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get 1000 µg/mL of the drug. The working standard solution of trapidil was prepared by diluting 0.3mL the above stock solution to 10 mL in a volumetric flask to get a 30 µg/mL of the drug solution. Further dilutions ranging from 10 to 50 µg/mL of the drug were prepared from the stock solution in 10 mL volumetric flasks for plotting the calibration curve.

RESULTS AND DISCUSSION

A mobile phase containing phosphate buffer (pH 3.0) and acetonitrile in the ratio of 45:55 v/v resulted in a good shaped peak for trapidil on Hypersil Symmetry C_{18} (100 X 4.6 mm; 5 µm) column. A flow rate of 0.9 mL /min was found to be optimum in the range of 0.8-1.0 mL/min and the drug was detected in the eluate at 221 nm.

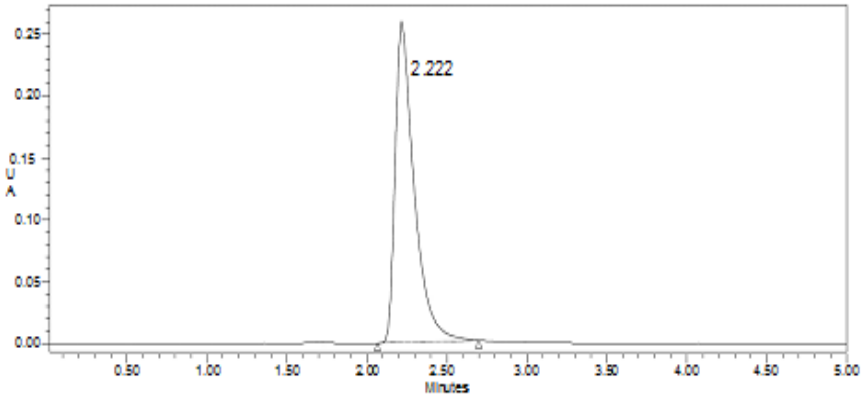

Figure 2: Chromatogram of Trapidil

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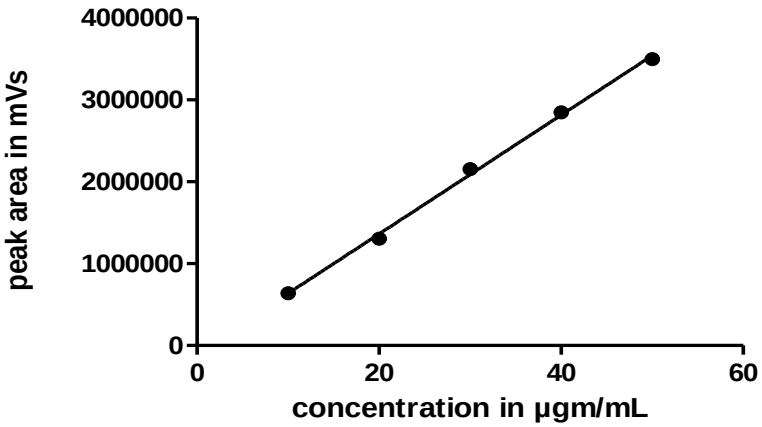

Figure 3: Calibration curve of Trapidil

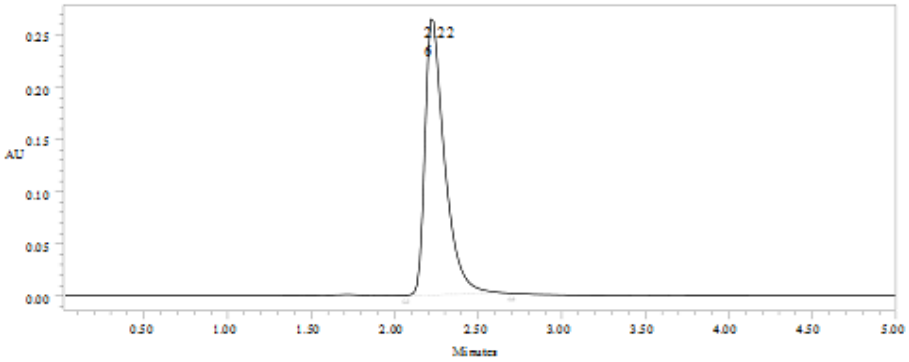

Figure 4: Chromatogram of Trapidil formulation

Table 1: Optimized chromatographic conditions of the proposed method

S. No.	Parameter	Value
1.	Mobile phase	Phosphate buffer (pH 3.0)-Acetonitrile (45:55 v/v)
2.	Diluent	
3.	Stationary phase	Symmetry C_{18}, (100 X 4.6 mm; 5 µm)
4.	Flow rate	0.9 mL/min
5.	Column temperature	25^0C
6.	Volume of injection	20 µL
7	Detection wavelength (λ_{max})	221 nm
8	Run time	5.00 min
9	Retention time	2.22 min

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Table: 2 Linearity range of Trapidil.

S.No.	Concentration (µg/mL)	Area of the peak	Statistical analysis
1	10 µg/mL	637476	Slope=72630 Intercept= ±1860 Correlation coefficient=0.9980
2	20 µg/mL	1306651	
3	30 µg/mL	2155424	
4	40 µg/mL	2846823	
5	50 µg/mL	3498769	

Table 3: Accuracy data of the proposed method

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2083661	5.0	5.03	100.7%	99.8%
100%	4136120	10.0	9.99	99.9%	
150%	6131030	15.0	14.8	98.7%	

Table 4: Precision of the method

Concentration Of Drug	Intra day precision			Inter day precision		
	Average peak area	S.D	%R.S.D	Average peak area	S.D	%R.S.D
30 µg/mL	2132350	11138.0	0.52	2170977	4294.4	0.20

Table 5: Results of the robustness study (flow rate)

S.No	Flow Rate (mL/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2889.3	1.7
2	0.9	2834.0	1.7
3	1.0	2650.9	1.6

Table 6: Results of the robustness study (mobile phase composition)

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2534.2	1.6
2	*Actual	2834.0	1.7
3	10% more	2401.3	1.5

***** Actual Mobile phase composition is 55:45 of ACN: Buffer

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Table 7: System suitability parameters of the proposed method

S.No.	Parameter	Result
1	Theoretical plates	2834
2	Tailing factor	1.7
3	HETP	$5.292x10^{-5}$
4	LOD (µg/mL)	0.015
5	LOQ (µg/mL)	0.048

Table 8: Recovery of Trapidil from formulations

S.No.	Formulation	Label claim (mg)	Amount found in mg (n=3)	% Recovery
1.	Trapilet	200	200	100 %

Under optimized chromatographic conditions, the retention time of trapidil was found to be 2.22 min. In the concentration over peak area plot of the drug, linearity was observed in the concentration range of 10-50 µg/mL. The regression equation of the curve was found to be Y=72630X+1860 (r = 0.998) where Y is the peak area and X is the concentration of trapidil (µg/mL). The intra-day and inter-day drug variation by the proposed method showed an RSD of less than 2% (0.52% and 0.20%), indicating that the method is quite precise. The drug content in the tablets was quantified using the proposed method of analysis. The corresponding recovery of trapidil was found to be 99.8%. The high percentage of recovery indicates that the proposed method is highly accurate. The mean amount of trapidil obtained in tablet dosage forms was 100%. The limit of detection and limit of quantitation of the drug were found to be 0.015 µg/mL and 0.048 µg/mL respectively indicating that the method is quite sensitive. The method shows good robustness for the minor variations in optimized chromatographic conditions. The tailing factor (1.7), number of theoretical plates (2834) and HETP (5.292×10^{-5}) obtained were shows the efficient performance of the column. No extra peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the determination of trapidil in pharmaceutical dosage forms. The values obtained for various validation parameters are within acceptance limits as per ICH guidelines[8]. Hence, this method can be easily and conveniently adopted for routine quality control analysis of trapidil in pure and its pharmaceutical dosage forms.

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