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DEVELOPMENT AND VALIDATION OF ULTRAVIOLET, RP-HPLC AND HPTLC METHODS FOR ESTIMATION OF TRAPIDIL BULK AND IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Quantitative estimation of Trapidil and its pharmaceutical dosage form by HPLC, HPTLC and UV spectroscopy methods was developed. In the RP-HPLC method, the drug was resolved using a mobile phase phosphate buffer: acetonitrile (30:70%v/v) with pH adjusted to 3.5 using phosphoric acid on C_{18} column in isocratic mode. The retention time of trapidil was found to be 3.195 min. In HPTLC method, the chromatograms were developed by using a mobile phase Methylene chloride: Methanol: ammonia (8.5:1:0.5 v/v) on precoated plate of silica gel $60F_{254}$ and quantified by densiometric absorbance mode at 312nm. The Rf value of Trapidil was 0.28. In the UV method, trapidil was quantified at 221nm in acetronitrile. Recovery studies of 98.8-101.14%, percentage relative standard deviation (%RSD less than 2%) and correlation coefficient (linearity range) that developed methods were accurate and precise. These methods can be employed for the routine analysis of tablets containing trapidil.

Key words: Trapidil, RP-PHLC, HPTLC, UV spectrophotometry, validation

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INTRODUCTION

Trapidil is chemically known as N, N-diethyl-5-methyl [1, 2, 4] triazolo [1, 5 alpha] pyrimidine-7-amine [1] (figure-1).

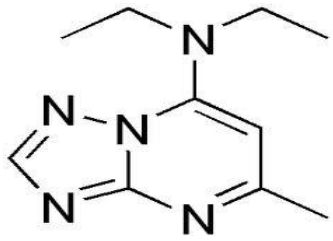

Figure1: Chemical structure of Trapidil.

Trapidil is used as vasodilator. Trapidil inhibits the cyclic AMP phosphodiesterase (PDE) activity. It increases the coronary sinus out flow and heart rate and decreases in systemic blood pressure in a dose dependent manner. Trapidil produces an increase in myocardial oxygen consumption but virtually, no change in coronary arteriovenous oxygen difference[2]. Literature survey revealed several analytical methods for the determination of Trapidil in bulk. The analytical technique published for determination of Trapidil and its metabolites in serum by HPLC and GC[3,4]. Determination of Trapidil in Human serum and urine by derivative UV spectroscopy after solid –phase extraction were reported[5&6]. No methods have been reported for determination of Trapidil in bulk and in tablet dosage form by UV, HPLC&HPTLC methods. The proposed research work describes the estimation of Trapidil in bulk and in tablet dosage form by UV, HPLC and HPTLC methods.

MATERIAL AND METHOD

Chemicals and Reagents

The bulk drug and tablet formulation of Trapidil was gifted from Ajanta Pharmaceuticals Mumbai. All solvents and reagents used were of HPLC (or) Analytical grade respectively. HPLC grade methanol, acetonitrile and chloroform used were of HPLC grade (E. Merck, Mumbai, India).

Instrumentation

UV Spectral measurements were recorded in Lab India spectrophotometer with 1cm quartz cells and photo multiplier tube detector were used. RP-HPLC was performed using water HPLC system (Milford, MA, USA) equipped with 600E HPLC pump, a 717 auto sampler and UV detector. The column was symmetry C_{18} Xterra (5µ, 4.6X150mm) used. The method was conducted using an isocratic technique. Data acquisition and processing was performed using

Empower software. HPTLC was performed in Cagmag HPTLC (Cagmag, Muttenz, and Switzerland) system, equipped with linomat V sample applicator twin trough plate development chamber, TLC scanner II with Cats soft ware.

UV-method

The stock solution was prepared by dissolving 10mg of TRP in 10ml of acetonitrile. The stock solution was further diluted with the solvent to obtained final concentration range (3-15 µg/ml). These solutions were used to calculate the linearity and the relative quantification of the tablets. About 20 tablets were weighed and powdered. A powder equivalent of 10mg of TRP was weighed accurately and transferred to a 10ml volumetric flask. The tablet powder was dissolved in acetonitrile and filtered through a whatman filter paper. The solution was further diluted and UV measurements were recorded.

RP- HPLC Method

The phosphate buffer was prepared by dissolving 7.0 grams of potassium dihydrogen phosphate in distilled water and made up to the volume 1000ml. The drug was resolved using a mobile phase of phosphate buffer: acetonitrile (30: 70%v/v) with pH adjusted to 3.5 using phosphoric acid, filtered using membrane filter and degassed. The flow rate was 0.6ml/min and the effluent was monitored at 306nm. A stock solution was prepared by dissolving 10mg of TRP in 10ml of mobile phase. The stock solution was further diluted with the mobile phase to obtain final concentration range (10-50 µg/ml). These solutions were used to calculate the linearity and the relative quantification of the tablets. About 20 tablets were weighed and powdered. A powder equivalent to 10mg was weighed accurately and transferred to 10ml volumetric flask. The tablet powder was dissolved in the mobile phase and filtered through a membrane filter under vacuum filtration. The sample was suitably diluted and used for the analysis. 20µl of standard and sample solutions were injected, under the specified conditions and the chromatogram was recorded. The amount of TRA present in tablet was calculated by peak area of sample with that of sample.

HPTLC Method

The drug was resolved using a mobile phase Methylene chloride: Methanol:ammonia (8.5:1:0.5 v/v) Chamber and plate saturation time was 30min, migration distance was 90mm and UV detection was carried out 312nm. A stock solution was prepared by dissolving 10mg of Trapidil in 10ml of mobile phase (1000µg/ml). The stock solution was further diluted with mobile phase to obtain final concentration range (2-10 µg/ml). The standard solutions were applied on the TLC plate. The chromatograms were developed. The developed chromatograms were evaluated by

scanning in densiometric mode at 312nm. The calibration curve was constructed by using peak area against concentration. The procedure was repeated for six times. The sample solution was prepared as that of HPLC method and filtered through whatman filter paper. Finally the sample was diluted to get a concentration (4µg/ml).The 4µl of sample and standard was applied on the TLC plate. The chromatogram was developed. The developed chromatograms were evaluated by scanning in densiometric mode at 312nm. The content of Trapidil in tablet was calculated by using the peak area of sample with that of standard.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back ground levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability [7].The LOD& LOQ were calculated as

$$LOD = 3.3\sigma/S \text{ and } LOQ = 10\sigma/S$$

Where σ is the standard deviation of the lowest standard concentration and S is the slope of standard curve.

Recovery studies

Recovery studies were carried out by adding known quantities of standard at different levels to the pre analyzed sample to study the linearity, accuracy and precision of the proposed method. The recovery[8] studies also reveals whether there is positive or negative influence on the quantification parameters by the additive usually present on dosage forms. The recovery study data are present in table.

RESULT AND DISCUSSION

In the UV method in which the drug, TRP shows a maximum absorbance at 221nm in acetonitrile. The spectrum was shown in figure-2. The linearity of TRA was found to be 3-15µg/ml and r^2=0.9998. The recovery values were 98.88-101.11% with percentage relative standard deviation (%RSD) of 1.2114.

In RP-HPLC method, the mobile phase was optimized with phosphate buffer: acetonitrile (30:70%v/v) with pH adjusted to 3.5 using phosphoric acid on C_{18} column in isocratic mode. Sharp peak was obtained with the retention time 3.195 min. The UV detection was carried out 306nm. An optimized chromatogram of TRP was shown in figure-3. The System suitability parameters (Table-1) were applied to a representative chromatograph to check various parameters such as with the USP requirements [9]. Linearity range of 10-50 µg/ml and r^2=0.999.

The recovery values were 99.33 -101.05% with percentage relative standard deviation (% RSD) of 0.9322.

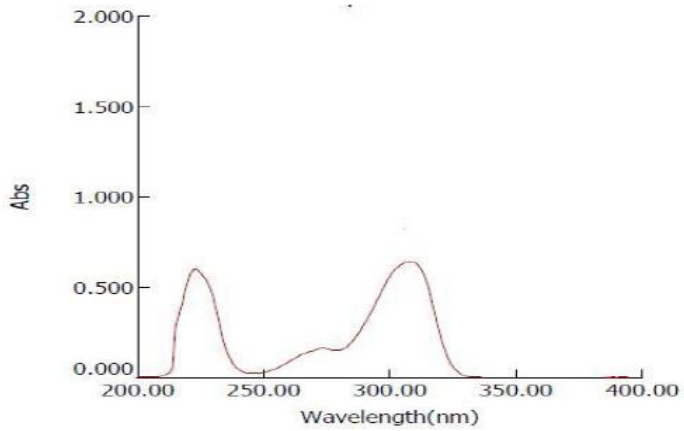

Figure 2: UV Spectrum of Trapidil

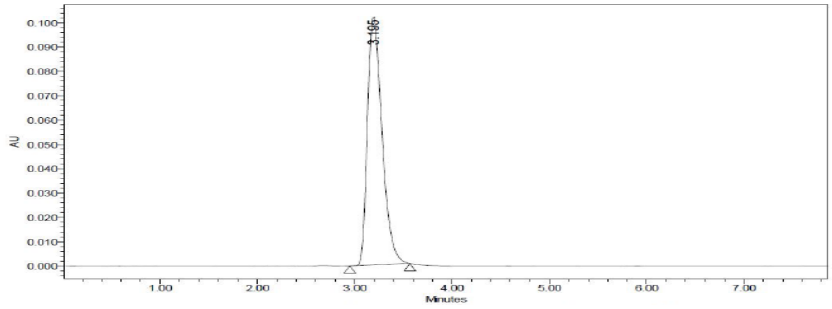

Figure 3: A Typical HPLC Chromatogram of Trapidil at 306nm

Table 1: System Suitability Parameters for HPLC

Parameters	Experimental Value	Limit as per USP
Tailing Factor	1.4	Less than 2
Asymmetric factor	1.0	Less than 2
No .of theoretical plates	2178.3	More than 2000
Capacity factor	3	2-10
HETP	0.0688	-
Theoretical plate per unit length	16.82	-

In HPTLC method, the chromatograms were developed by using a mobile phase methylene chloride: methanol: ammonia (8.5:1:0.5 v/v) on precoated plate of silica gel $60F_{254}$ and quantified by densiometric absorbance mode at 312nm.The optimized chromatogram was shown in figure-4. The Rf value of 0.28 with a linearity range of 2 to10 µg/ml and r^2 =0.9993. The recovery values[10] were 99.44 -101.14% with %RSD of 1.1124.

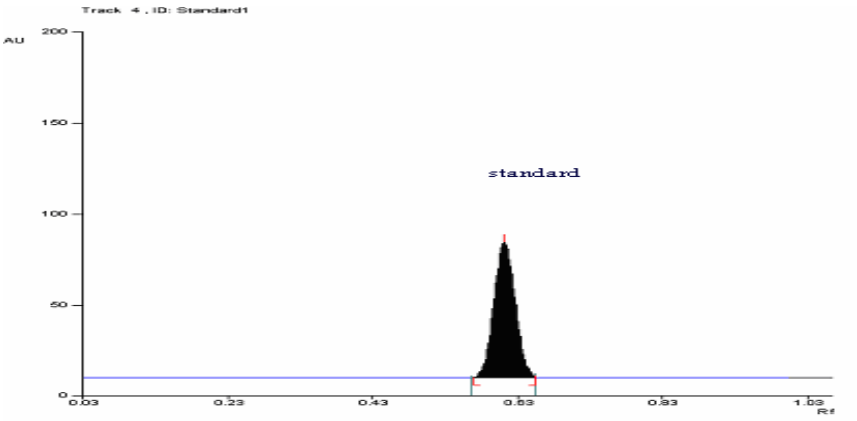

Figure 4: **A Typical HPTLC Chromatogram of Trapidil at 312nm**

Table 2: Summary of Validation Parameters for Trapidil by the Proposed UV, RP-HPLC and HPTLC

Validation Parameters	UV	HPLC	HPTLC
Beer's law limit	3-15µg/ml	10-50µg/ml	2-10 µg/m
Correlation coefficients r^2	r^2=0.999	r^2=0.999	r^2 =0.9993
Regression equation	Y=0.056X-0.007	Y=132848X+3345	Y=3965.88X+279.30
Slope	0.056	132848	3965.88
Intercept	0.007	3345	279.30
LOD	0.216 µg/ml	0.014 µg/m	0.0034 µg/ml
LOQ	0.655 µg/ml	0.0465 µg/ml	0.0105 µg/ml
Precision			
Interaday (% RSD)	0.6612	0.1715	0.5444
Intraday (% RSD)	0.7735	0.5223	0.9823
Accuracy (% RSD	1.2114	0.9322	1.1124

Table 3: Recovery analysis for Trapidil by the Proposed UV, RP-HPLC and HPTLC

Methods	Amount present µg/ml	Amount added µg/ml	Amount found µg/ml	Amount received µg/ml	%Recovery	SD	%RSD
UV	9.02	1.8	10.82	1.78	98.88		
	9.02	3.6	12.62	2.57	100.83	1.2147	1.2114
	9.02	5.4	14.42	5.46	101.11		
HPLC	30.0	6.0	36.0	5.97	99.33		
	30.0	12.0	42.0	12.05	100.83	0.9360	0.9322
	30.0	18.0	48.0	17.95	101.05		
	6.03	1.2	7.23	1.19	99.44		
HPTLC	6.03	2.4	8.43	2.43	99.05	1.111	1.1124
	6.03	3.6	9.63	3.57	101.14		

*Mean of six observations

In these three methods the validation parameters results were shown in Table-2. Recovery values were shown in Table-3. All the method validation parameters are well within limits as specified in the ICH guidelines Q2B [11].The validation parameters results were shown in Table-3.

CONCLUSION

The UV, HPLC and HPTLC methods developed for Trapidil shows good precision and accuracy. The low %RSD values in the recovery studies for these method shows that there is no interference due to excipients used in the formulation. Hence it was concluded that the developed methods are simple, precise, accurate and rapid for the analysis of Trapidil in pure and in tablet dosage form. Thus the developed methods can be adopted for the routine analysis of Trapidil in bulk and tablet dosage form.

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