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sum. The molecular structure of Tadalafil is similar to that of cGMP and act as a competitive binding agent of PDE5 in the corpus cavernosum, resulting in more cGMP and better erections. Without sexual stimulation, and therefore lack of activation of the NO/cGMP system, Tadalafil should not cause an erection. Other drugs that operate by the same mechanism include tadalafil (Cialis®) and vardenafil (Levitra®).

Tadalafil is metabolised by hepatic enzymes and excreted by both the liver and kidneys. If taken with a high-fat meal, there may be a delay in absorption of Tadalafil and the peak effect might be reduced slightly as the plasma concentration will be lowered.

Fixed dose containing Tadalafil citrate (10 mg) is available in the market as tablet. The present paper describes a simple, precise and accurate HPTLC method for determination of Tadalafil Citrate from tablet.

Chemicals and reagents

The formulation was procured from pharmacies. Standards were from reputed research center. Toluene, Ethyl acetate, Methanol and Glacial acetic acid used were analytical grade. All dilutions were performed in standard volumetric flasks.

EXPERIMENTAL

Instrument

A camag, Linomat IV sample applicator was used. Camag Twin trough glass chamber (20×10cm) was used for development of plates. And Camag TLC scanner III equipped with cats 3 Version software was used for

interpretation of data.

Standard preparation

Accurately weigh 25mg of standard Tadalafil citrate was taken in 25mL volumetric flask. This was dissolved in minimum quantity of methanol and made up to volume to get a concentration of 1000 µg/mL (Solution A).

Accurately weigh 10mg of standard Sildenafil citrate was taken in 10mL volumetric flask. This was dissolved in minimum quantity of methanol and made up to volume to get a concentration of 1000µg/ml (Solution B).

Chromatographic conditions

Application: 10µl of standard were applied as bands of 7mm width using Camag Linomat IV- Applicator, and developed in Camag twin trough chamber.

Mobile Phase : Toluene: Ethyl acetate: Methanol: Glacialaceticacid (5:3.5:0.8:0.2) v/v
Saturation time : The chamber was saturated with mobile phase for 10mins
Migration distance : 8cm
Wavelength of detection : 254nm using Camag TLC Scanner II with cats3 software

A typical HPTLC chromatogram for determination of Tadalafil Citrate from pharmaceutical formulation is shown in figure 2.

RESULTS AND DISCUSSION

Linearity

Into a series of 10mL volumetric flasks, varying

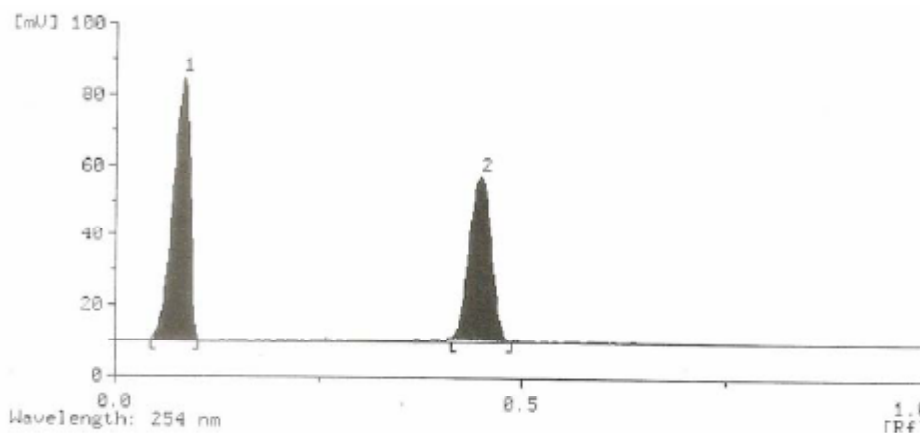


Figure 2: HPTLC chromatogram for Tadalafil citrate with respect to the internal standard Sildenafil citrate: - 1. Sildenafil Citrate; 2. Tadalafil Citrate

TABLE 1: Analytical performance data

Linearity	100 to 220 $\mu\text{g/mL}$
Slope (m)	0.0058
Intercept (b)	0.0716
Correlation coefficient (R)	0.9993

TABLE 2: Assay results

Drug	Labeled claim (mg)	Drug found in mg	%COV ^a	% Assay
Tadalafil citrate	150	150.52	1.13	100.34

^aCoefficient of variation

TABLE 3: Recovery table

Initial conc. $\mu\text{g/mL}$	Conc. added $\mu\text{g/mL}$	Total conc. $\mu\text{g/mL}$	Conc. found $\mu\text{g/mL}$	RSD (%) N=7	Recovery (%)
150	0	150	150.27	0.88	100.18
150	15	165	165.56	1.06	100.34
150	30	180	179.59	0.80	99.76
150	45	195	194.38	0.88	99.68

concentrations from 100 to 220 $\mu\text{g/mL}$ of Tadalafil citrate were transferred and in each flask, 2.5ml of stock pure drug solution of Sildenafil citrate was added and the volume was made up to the mark with methanol from their respective stock solutions. The above concentrations were applied on the chromatographic plates. The plate was developed using mobile phase comprising of Toluene: Ethyl acetate: Methanol: Glacialaceticacid in the volume ratio (5:3.5:0.8:0.2) in twin trough chamber to a distance of 8cm. The plate was then removed from chamber and air-dried. The plate was then scanned and quantified at 254nm. Peak area ratio of Tadalafil citrate to Sildenafil citrate was calculated and respective calibration curve were plotted against concentration of drug and peak area ratio of drug to internal standard. A linear relationship was observed for Tadalafil citrate with respect to the internal standard Sildenafil citrate. A HPTLC chromatogram is given in figure 2 and the linearity data are given in TABLE 1.

Assay

Twenty tablets were weighed and average weight was calculated. The tablets were powdered and weight equivalent to 150mg of pure standard Tadalafil citrate was taken in a 100mL volumetric flask. To this volumetric flask 25 mg of pure standard Sildenafil citrate was added and dissolved in minimum amount methanol. The solution was filtered through Whatman no. 41. the filtrate was collected in 100mL volumetric flask and then diluted up to the mark with methanol. Further

this solution was diluted with methanol to get concentration 150 $\mu\text{g/mL}$ of Tadalafil citrate and 250 $\mu\text{g/mL}$ of internal standard Sildenafil citrate. This sample solution 10 μL was spotted in triplicate along with standard solution on HPTLC plate precoated silica gel 60F254 under the optimized chromatographic conditions. Peak area ratio of Tadalafil citrate to Sildenafil citrate was estimated using calibration curve method. Result of assay experiment is given in TABLE 2.

Recovery

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation, and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method. The recovery of the added standard was studied at three different levels, each being analysed in a manner similar to as describe for assay. Each set of recovery of added standard was calculated. The results of recovery experiment are tabulated in TABLE 3.

DISCUSSION AND CONCLUSION

The method was a normal phase HPTLC method. It makes use of a silica gel 60F₂₅₄ stationary phase precoated on aluminium sheet. The mobile phase comprises Toluene: Ethyl acetate: Methanol: Glacial Acetic Acid in the volume ratio of (5:3.5:0.8:0.2) which gives good separation between Tadalafil citrate ($R_f=0.45$) and Sildenafil citrate ($R_f=0.10$). The linearity range of Tadalafil citrate was found to be 100 to 220 $\mu\text{g/mL}$. And coefficient of variation was found to be 0.9993. The limit of detection and quantification for Tadalafil citrate was found to be 20 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$ respectively. The average recovery was found to be 99.995%, which shows that method is free from interference from excipients present in the formulation. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

This High performance thin layer chromatographic method for determination of Tadalafil citrate in fixed dosage form was found to be accurate and precise. The method can therefore be used for routine quality-control analysis of Tadalafil citrate in such formulations.

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