October 2008



Volume 7 Issue 9

nalytical CHEMISTR An Indian Journal

Trade Science Inc.

Full Paper

ACAIJ, 7(9) 2008 [729-732]

## Determination of tadalafil citrate by HPTLC in pharmaceutical preparations

R.U.Kanchan\*, Shikha M.N.Roy, Kiran Mangaonkar, A.Desai <sup>1</sup>Department of Chemistry, G.N.Khalsa College, Matunga, Mumbai-400019 (INDIA) Telephone: 09821006808 E-mail: rukanchan@rediffmail.com Received: 23<sup>rd</sup> August, 2008 ; Accepted: 28<sup>th</sup> August, 2008

## ABSTRACT

Using sildenafil citrate as internal standard. The analytes were resolved on HPTLC plates (Merck) Silica gel  $60F_{254}$  by using mobile phase Toluene: Ethylacetate: Methanol: Glacialaceticacid (5:3.5:0.8:0.2) v/v, with chamber saturation 10min. The plate was developed up to 8cm and air-dried. The plate then was scanned and quantified at 254nm. Sildenafil citrate was used as an internal standard for HPTLC method. The linearity of Tadalafil citrate is in the range of 100 to 220 µg/mL. The limit of detection and limit of quantification for Tadalafil citrate were found to be  $20\mu g/mL$ ,  $50\mu g/mL$  respectively. The proposed method is accurate, precise and rapid for determination of Tadalafil citrate. © 2008 Trade Science Inc. - INDIA

#### INTRODUCTION

Tadalafil citrate is a drug used to treat male erectile dysfunction (importance). It works by inhibiting an enzyme known as PDE5. The empirical formula for Tadalafil is  $C_{22}H_{19}N_3O_4$ , and its official organic name is (6R, 12aR)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino[1,2:1,6] pyrido [3,4-b] indole-1, 4-dione. The molecular weight is 389.41. The structure of the drug is shown in figure 1.

Part of the physiological process of erection involves

## KEYWORDS

ICH Guidelines; Validation; HPTLC; Pharmaceutical preparations; Tadalafil citrate; Sildenafil citrate.

the parasympathetic nervous system causing the release of nitric oxide (NO) in the corpus cavernosum of the penis. NO binds to the receptors of the enzyme guanylate cyclase, which results in, increased levels of cyclic guanosine monophosphate (cGMP), leading to smooth muscle relaxation (vasodilation) in the corpus cavernosum, resulting in increased inflow of blood and an erection.

Tadalafil is a potent and selective inhibitor of cGMP specific phosphodiesterase type 5 (PDE5), which is responsible for degration of cGMP in the corpus caverno



Figure 1: Structures of tadalafil citrate and sildenafil citrate

# Full Paper

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 (B) and vardenafil (Levitra(B)).

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

Analytical CHEMISTRY

An Indian Journal

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  $1000 \,\mu\text{g/mL}$  (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  $1000\mu$ g/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.

v/v
mo
er
e

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





	TABI	LE 1: Analyt	ical perforn	nance da	ita		
Lineari	ty		1	100 to 220 µg/mL			
Slope (n	n)			0.0058			
Intercep	t (b)			0.0716			
Correlation coefficient (R)				0.9993			
		TABLE 2:	Assay resu	ılts			
Drug		Labeled	Drug four	nd %CO	$DV^a$		
		claim (mg	) in mg $150.52$	in mg			
Tadalafil citrate		150	150.52	1.1	3 100.34		
<sup>a</sup> Coefficier	nt of va	riation					
		TABLE 3:	Recovery ta	ble			
Initial	Con	c. Total	Conc.	RSD	Recovery (%)		
conc.	adde	d conc.	found	(%)			
µg/mL	µg/m	L µg/mL	µg/mL	N=7			
150	0	150	150.27	0.88	100.18		
150	15	165	165.56	1.06	100.34		

150 45 195 194.38 99.68 0.88 concentrations from 100 to 220µg/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

150

30

180

179.59

0.80

99.76

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 100mLvolumetric 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  $150\mu g/mL$  of Tadalafil citrate and  $250\mu g/mL$  of internal standard Sildenafil citrate. This sample solution  $10\mu L$  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  $60F_{254}$  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 ( $R_{e}=0.45$ ) and Sildenafil citrate ( $R_e = 0.10$ ). The linearity range of Tadalafil citrate was found to be 100 to 220µg/mL. And coefficient of variation was found to be 0.9993. The limit of detection and quantification for Tadalafil citrate was found to be 20µg/mL and. 50µg/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 qualitycontrol analysis of Tadalafil citrate in such formulations.



REFERENCES

- [1] ICH; Centre for Drug Evaluation and Research, Rockville, (1996).
- [2] L.R.Snyder, J.J.Kirland, J.L.Glajch; 'Practical HPLC Method Development', 2<sup>nd</sup> edition, John Wiley and Sons, Inc., U.S.A., (1997).
- [3] S.Thomas Forgue; Beverley E.Patterson, Alun W. Bedding, Christopher D.Payne, Diane L.Phillips, Rebecca E.Wrishko, Malcolm I.Mitchell; Br.J.Clin. Pharmacol., 61(3), 280-288 (2006).
- [4] Berniati Tampubolon, Hosiana, Sumarlik, Endang, Dwi Saputra, Setiawan, Cholifah, Siti, Farina Kartinasari, Wiwin, Indrayanto, Gunawan; Journal of Liquid Chromatography and Related Technologies, 29(18), 2753-2765 (2006).
- [5] Quanyun A.Xu, Lawrence A.Trissel, G.V.Sager, P.V.Rao; The Antiseptic., **99(8)**, 282-3 (**2002**).
- [6] N.D.Dinesh, B.K.Vishukumar, P.Nagaraja, N.M. Made Gowda, K.S.Rangappa; Stability indicating RP-LC determination of sildenafil citrate (Viagra) in pure form and in pharmaceutical products.