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Short communication

Determination of tadalafil in pharmaceutical preparation by HPLC using monolithic silica column

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Abstract

The simple, reliable and reproducible HPLC and extraction methods were developed for the analysis of tadalafil in pharmaceutical preparation. The column used was monolithic silica column, Chromolith Performance RP-18e (100 mm × 4.6 mm, i.d.). The mobile phase used was phosphate buffer (100 mM, pH 3.0)-acetonitrile (80:20, v/v) at the flow rate of 5 mL min^{-1} with UV detection at 230 nm at ambient temperature. Extraction of tadalafil from tablet was carried out using methanol. Linearity was observed in the concentration range from 100 to 5000 ng mL^{-1} for tadalafil with a correlation coefficient (R^2) 0.9999 and 100 ng mL^{-1} as the limit of detection. The values of linearity range, correlation coefficient (R^2) and limit of detection were 50–5000 ng mL^{-1}, 0.9999–50 ng mL^{-1}, respectively for sildenafil. Parameters of validation prove the precision of the method and its applicability for the determination of tadalafil in pharmaceutical tablet formulation. The method is suitable for high throughput analysis of the drug.
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Keywords: Tadalafil; Sildenafil; Monolithic column; Pharmaceutical preparation

1. Introduction

Erectile dysfunction (ED), inability to achieve a penile erection sufficient for satisfactory sexual performance, is estimated to affect many men world wide [1–3]. ED is more common in advanced age [3–5] and related to hypertension or diabetes mellitus or use of certain pharmacological agents e.g. antihypertensives and antihypertensive [1]. ED has been treated by the drugs that inhibit the enzyme phosphodiesterase type 5 (PDE5) activities. Sildenafil [6] was first drug for this purpose and another remarkable achievement in this direction is the introduction of second phosphodiesterase inhibitor called tadalafil, which was launched in February 2003 [7]. Tadalafil is chemically known as pyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-,(6R-trans)-(6R-,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl]-pyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (Fig. 1). Tadalafil is a selective phosphodiesterase type 5 inhibitor, which is used to treat mild to severe ED in man. Drug testing is an integral part of pharmaceutical analysis and routine quality control monitoring of drug release characteristics. To the best of our knowledge no report has been published on the analysis of tadalafil in pharmaceutical preparations.

The speed and economic analysis is becoming increasingly important in many application areas of HPLC including pharmaceutical analysis in order to increase throughput and reduce costs. Recently, a special type of silica based column of high speed has been introduced into the market, which is called monolithic column [8,9]. Some reviews [10,11] have been published claiming the fast and economic analysis using

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this column for a variety of compounds. Therefore, attempts have been made to analyze tadalafil in the tablet formulation using the monolithic silica column and the results are presented herein.

2. Experimental

2.1. Chemicals and reagents

Standard tadalafil (Fig. 1) was obtained from Eli Lilly and Company, USA. Cialis® tablet (containing 20 mg of tadalafil), manufactured by Eli Lilly and Company, USA, was purchased from local market. Sildenafil citrate (Fig. 1), used as internal standard (IS), was a gift from Pfizer Central Research (Sandwich Kent, NJ). Standard solutions (50 $\mu g\,mL^{-1}$) of the individual and the mixture of tadalafil and sildenafil were prepared in methanol. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile, ethanol and methanol of HPLC grade and *o*-phosphoric acid of A.R. grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Sodium dihydrogen orthophosphate ($NaH_2PO_4{\cdot}2H_2O$) of A.R. grade was obtained from BDH Limited (Poole, England). Phosphate buffers were prepared by the standard methods. For phosphate buffer preparation of 100 mM and pH 3.0, 1.56 g of $NaH_2PO_4{\cdot}2H_2O$ was weighed and dissolved in 100 mL purified water. pH of this solution was adjusted using *o*-phosphoric acid with the help of a pH meter. Similarly, buffers of different concentrations and pHs can be prepared by this methodology.

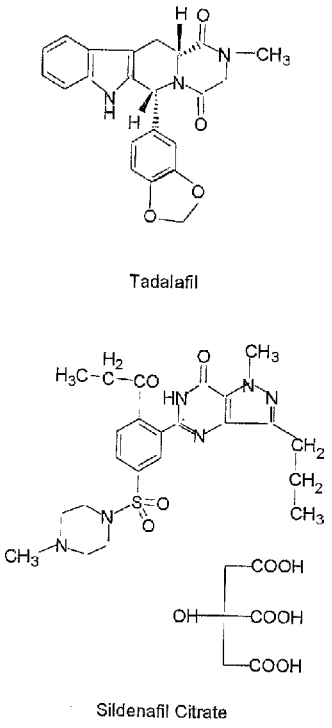

Fig. 1. Chemical structures of tadalafil and sildenafil.

2.2. Instruments used

HPLC system consisting of Waters solvent delivery pump (model 510, Milford, Massachusetts, USA), Waters injec-

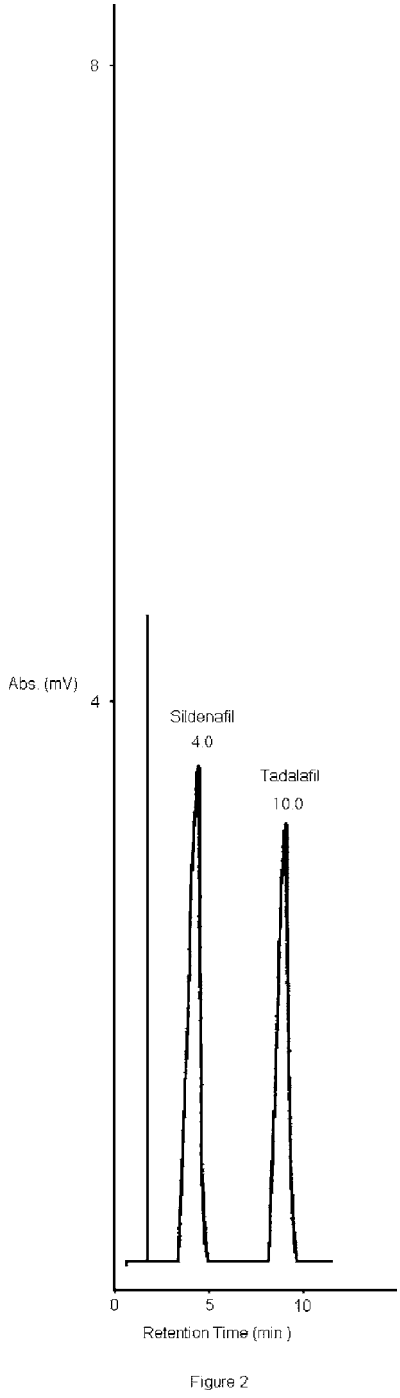

Fig. 2. Chromatograms of tadalafil and sildenafil extracted from tablet on monolithic silica column (100 mm × 4.6 mm, i.d.) using phosphate buffer (100 mM, pH 3.0)-acetonitrile (80:20, v/v) as the mobile phase at 5 mL min^{-1} flow rate with UV detection at 230 nm.

Table 1
Chromatographic and precision data of tadalafil and sildenafil

Compounds	k_1	α	R_s	S.D.	Correlation confidence	Coefficient level (%)
Tadalafil						
Standard	33.01	2.62	2.0	±0.05	0.9999	99.7
Tablet mixture	33.01	2.62	2.0	±0.05	0.9999	99.5
Sildenafil						
Standard	12.61	–	–	±0.05	0.9999	99.8
Tablet mixture	12.61	–	–	±0.05	0.9999	99.7

k: capacity, α = separation and R_s: resolution factors. Column: monolithic silica column (100 mm × 4.6 mm, i.d.). Mobile phase: phosphate buffer (100 mM, pH 3.0)-acetonitrile (80:20, v/v). Flow rate: 5 mL min^{-1}. Detection: UV at 230 nm. Temperature: 23 ± 1 °C; n = 7. S.D.: standard deviation of R_s.

tor (model WISP 710B), Waters tunable absorbance detector (model 484) and Waters integrator (model 740) was used in this study. Monolithic silica column, Chromolith Performance RP-18e (100 mm × 4.6 mm, i.d.) was purchased from Merck KgaA (Darmstadt, Germany). pH meter used was Orion Research (model 611), Orion Research Incorporation, USA. Millipore Milli-Q (Bedford, M.A., U.S.A.) system was used for deionised water.

2.3. Chromatographic conditions

An aliquot of 20 μL of individual and the mixture of tadalafil and sildenafil was injected on to a HPLC system separately and respectively. The mobile phases used in this study was phosphate buffer (100 mM, pH 3.0)-acetonitrile (80:20, v/v) with a flow rate of 5 mL min^{-1}. The mobile phase was filtered and degassed before use. The mobile phase was filtered using Durapore membrance filters 0.22 μm GVHP (Millipore, Ireland). The chart speed was kept constant at 0.1 cm min^{-1}. All the experiments were carried out at 23 ± 1 °C. The detection was carried out at 230 nm. The chromatographic parameters, such as capacity (k), separation (α) and resolution factors (R_s) were calculated. The identification of the separated tadalafil and sildenafil was confirmed by running the chromatograms of the individual compounds under identical chromatographic conditions. The internal addition method was also applied for the confirmation of both the molecules in the tablet extract.

2.4. Extraction of tadalafil from tablet

To avoid any interference in HPLC separation, the sugar part (outer layer) of the tablet was removed by sculpture and the inner part of the tablet was ground to a fine powder. Fifty milligram of this powder was weighed and mixed with 2 mg of sildenafil (internal standard) and the combined powder was extracted with 5 mL methanol by sonicating at 80 °C and then cooled. Methanol was filtered and the residue again was extracted with methanol (5 mL) twice as described above. Methanol extracts were combined together and diluted to 10 times with methanol and used for chromatographic studies. Besides, the extraction of tadalafil from tablet was also carried out using ethanol, acetone and diethyl ether.

3. Results and discussion

To know the percentage recovery of tadalafil from tablet sildenafil was used as the internal standard because the later has the similar properties with tadalafil. Therefore, the separation of sildenafil and tadalafil is essential, and the capacity (k), separation (α) and resolution (R_s) factors for these two compounds in standard and tablet extract are calculated and given in Table 1. The chromatograms of tadalafil and sildenafil in tablet extract are shown in Fig. 2, which indicate a good base line separation. The chromatograms of the individual tadalafil and sildenafil were also recorded under the identical chromatographic conditions. Tadalafil and sildenafil in tablet extract were identified by comparing their retention times with the retention times of the individual compounds. The confirmation of tadalafil and sildenafil was carried out by the internal addition method. The order of the elution was sildenafil followed by tadalafil (Fig. 2). The calibration curves were plotted for both compounds and were used to determine their concentrations in pharmaceutical preparation. The calibration curves were prepared by using 100–5000 and 50–5000 ng mL^{-1} concentrations of tadalafil and sildenafil, respectively.

To optimize the chromatographic conditions, various combinations of phosphate buffer-acetonitrile (90:10, 95:5, 70:30, 60:40, 50:50, 40:60, 30:70, 80:20, 90:10 and 95:5) and water-acetonitrile (90:10, 95:5, 70:30, 60:40, 50:50, 40:60, 30:70, 80:20, 90:10 and 95:5) were tested. Besides, buffers of different concentrations and pHs (10–500 mM of 2.0–8.0 pHs), alone or with acetonitrile and methanol were also used. At higher concentration of acetonitrile the values of separation and resolution factors were 0.80 and 0.50, respectively. Again the values of these factors were very poor when using phosphate buffer of pH greater than 3.0. The resolution

Table 2
Regression analysis data for the extraction (% recovery) of tadalafil and sildenafil

Compounds	Recovery (%)	S.D.	Correlation coefficient (R^2)	Confidence level CL (%)
Tadalafil	99.5	±0.08	0.9999	99.4
Sildenafil	99.8	±0.08	0.9999	99.5

S.D.: standard deviation, n = 7.

Table 3
The intra- and inter days data for tadalafil and sildenafil

Parameter	Tadalafil		Sildenafil	
	Intra-day (n = 3)	Inter-day (n = 3)	Intra-day (n = 3)	Inter-day (n = 3)
Correlation coefficient	0.9998	0.9998	0.9999	0.9999
Confidence limit	99.3	99.2	99.6	99.6
S.D.	±0.05	±0.05	±0.05	±0.05

S.D.: standard deviation, n = 7.

was incomplete using different combinations of phosphate buffer and methanol. The peaks of tadalafil and sildenafil were broad using different concentrations of water and acetonitrile. However, at higher concentration of acetonitrile in water-acetonitrile mixture both the drugs eluted at the same retention time. It is interesting to note that the detection was poor at low concentration of acetonitile in all the mobile phases as water is good UV radiation absorbing solvent. Briefly, as a result of extensive experiments the optimized HPLC conditions were developed and reported herein.

The capacity factors for tadalafil and sildenafil were 33.01 and 12.61 with 2.62 and 2.0 as the separation and resolution factors for tadalafil respectively. It is interesting to note that the values of the chromatographic parameters (Table 1) are similar for the standard solution and tablet extract, which indicates the robustness of HPLC method. The quantitative analysis of tadalafil in Cialis® tablet was determined by comparing the peak area of tadalafil with the peak area of standard tadalafil. It has been observed that the recovery of tadalafil from the tablet was 99.5%. The efficiency of the extraction methodology was ascertained by using sildenafil as the internal standard. pH of the reported mobile phase was 3.0 and at this pH both tadalafil and sildenafil exist as ammonium cations and, hence, these molecules interact with the silanol groups through some electrostatic forces. Besides, the dispersion forces, hydrogen bonding, Van der Waal forces and steric effect are also playing some role in the separation phenomenon of the reported compounds on monolithic silica column.

The calculated recoveries of tadalafil and sildenafil were 99.5 and 99.8, respectively (Table 2). Methanol effectively extracted tadalafil and sildenafil from tablet powder and gave satisfactory recoveries for both tadalafil and sildenafil. On the other hand, the percentage recoveries were only 60, 66, 70 and 75 % using diethyl ether, ethyl acetate, ethanol and acetone respectively. The polarities of these solvents are in the order of diethyl ether < ethyl acetate < acetone < ethanol < methanol. Therefore, the maximum percentage recoveries of tadalafil and sildenafil were obtained using methanol. Extraction of tadalafil from tablet was also tried at 50–60 °C (below boiling point of methanol) using methanol as the extraction solvent but the percentage recovery was only 85–90%. Therefore, methanol at 80 °C was used as an extracting solvent for tadalafil from Cialis® tablets as this achieved higher recovery as shown in Table 1.

3.1. Validation of the method

The validation of the developed method was ascertained by carrying out seven sets (n = 7) of the chromatographic and extraction procedures under identical conditions. The regression analysis was carried out using Microsoft Excel program and the results are given in Tables 1 and 2 for the chromatographic and extraction procedures respectively. It is clear from Table 1 that the standard deviation (S.D.) and correlation coefficients (R^2) were ±0.05 and 0.9999, respectively while the confidence levels ranged from 99.5 to 99.8. Similarly, Table 2 shows that the values of standard deviation and correlation coefficients are ±0.08 and 0.9999, respectively while the values of confidence level were 99.4 and 99.5 for tadalafil and sildenafil extraction respectively. The correlation coefficients for calibration curves were higher than 0.999 as determined by least square analysis. The low detection limits for tadalafil and sildenafil were 100 and 50 ng, respectively. The inter- and intra-days (7 days) analysis assays were also carried out and the results indicated no remarkable variations in their concentrations. The regression data of the precision are given in Table 3. The values of correlation coefficients were from 0.9998 to 0.9999 while the confidence limit ranged from 99.2 to 99.6. The values of standard deviation were ±0.05 for both tadalafil and sildenafil.

4. Conclusion

The developed chromatographic and extraction methods are simple, reliable and reproducible for the analysis of tadalafil in pharmaceutical formulation. The reported method can be used successfully for effective qualitative and quantitative analysis of tadalafil in other pharmaceutical preparations.

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