High Performance Liquid Chromatographic Method for Determination of Tadalafil in Tablets and Wastewater

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ABSTRACT

Objective: A reverse phase high performance liquid chromatographic method (RP-HPLC) has been developed for the determination of tadalafil in the pharmaceutical formulations and environmental wastewater samples.

Method: Different analytical columns with various stationary phases were tested. Good separation was achieved using supelcoC18 column (25cm x 4.6 mm. 5 µm) . The mobile phase (methanol: water: Triethylamine) , pH adjusted to 4.0 with dilute phosphoric acid was pumped at a flow rate of 1.3 ml/min in the ratio of 60:38:2 and the peaks were detected at 220 nm.

Results: Linearity was obtained in the concentration range of 0.0 4-0.28 mg/ml .The RSD was found to be less than 1% indicating the method is precise and the recovery was100±1.25% indicating the method is accurate

Conclusion: The proposed HPLC method may be used for determining tadalafil in pure drug samples , pharmaceutical dosage forms and environmental wastewater samples.

الخلاصة

الهدف : تم اختبار طريقة كروماتوغرافيا السائل ذات الأداء العالي لتقدير عقار التدالافيل في حالته النقية وفي بعض مستحضراته الصيدلانية(الحبوب)وفي نماذج من المياه الصناعية المطروحة

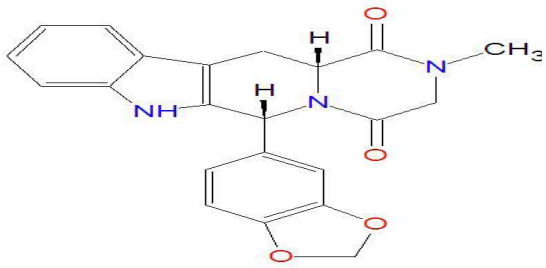
طريقة العمل : تم اختبار عدة أعمدة ووجد بان أفضل عملية فصل هي باستخدام عمود س18 (25سم×4.6 5. مايكرومتر) وقد تم استخدام الطور المتحرك المتكون من(الميثانول- الماء-ثلاثي اثيل امين)وبنسبة (60-38-2) وتم تثبيت ألداله الحامضية على 4 باستخدام حامض الفسفوريك المخفف وبسرعة جريان 1.3 مل\دقيقة وتم القياس عند الطول الموجي 220 نانومتر

النتائج: أمكن قياس الكميات 0.04-0.28 ملغم\مل ووجد إن الانحراف القياسي النسبي للطريقة اقل من1% مما يدل على ان الطريقة ذات توافق جيد

الاستنتاج: تم استخدام ألطريقه لتقدير التدالافيل بشكله النقي وفي مستحضر الحبوب وفي المياه الصناعية المطروحة.

Tadalafil is a potent and selective phosphodiesterase-5 (PDE-5) inhibitor a secondary messenger for the smooth muscle relaxing effect of nitric oxide which plays an important role in the vasodilation of erectile tissues. [1]

Tadalafil chemically know as; Hydro-2-methyl-6-[3,4-(methylene dioxy) phenyl] pyrozino-[1',2':1,6] pyrido[3,4-b] indole-1,4-dione. Popularly known as Cialis and having the following structural formula, Fig(1).



$C_{22}H_{19}N_3O_4$= 389.409

Fig(1):Chemical structure of tadalafil.

Extensive literature survey revealed that the determination of the drug in pure and dosage forms are not officially in any pharmacopoeia therefor, require much more investigation. There are several methods for determination of tadalafil such as HPLC[2-5], HPLC-EIMS[6], HPTLC[7] , capillary electrophoresis[8], spectrophotometry[9,10], densitometry[11], by electro spray tandem mass spectrometry (ESI-MS-MS)[12], uv-spectrophotometry[13], atomic emission and atomic absorption spectrometry[14]. High performance liquid chromatography (HPLC) can be used for determination of drugs and for purposes of control throughout the entire manufacturing process of drugs. as well as quality control of the finished product. It has the advantages of being sensitive, selective, rapid, accurate and reproducible. The present paper reports the development of a new high performance liquid chromatographic (HPLC) method for determination of tadalafil in pharmaceutical preparations and environmental wastewater samples

Experimental

Apparatus

Chromatographic system consisted of an Shimadzu HPLC model LC-20AT with UV detector model SPD-20A,pump LC-20 AT ,degasser model DGU -20A and C_{18} supelco column (25cm×4.6mm),5 µm particle size .

Reagents

All chemicals used were of analytical or pharmaceutical grade, HPLC grade methanol was used throughout . and tadalafil standard material was provided from the state company for pharmaceutical industries (NDI) Mosul-Iraq.

Preparation of standard solutions: A standard stock solution of tadalafil (0.4 mg/ml) was prepared in methanol, Working standard solutions in a range of (0.04-0.28 mg/ml) were prepared by dilution from this stock solution.

Preparation of mobile phase: The mobile phase was prepared by mixing methanol, water, triethylamine in the ratio of 60: 38: 2 %v/v/v, pH adjusted to 4.0 with dilute phosphoric acid

HPLC method for determining tadalafil

A series of standard solution containing 0.04-0.28 mg/ml of tadalafil and the sample solution of pharmaceutical preparations and water samples were applied respectively. 20µl aliquot of each solution was injected on to the column in a duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of tadalafil. The

concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from the concentration and peak area data.

Procedure for pharmaceutical preparations(tablets)

To minimize a possible variation in the composition of the tablet, the mixed content of 20 tablets were weighed and grounded, then the powder equivalent to 100mg of tadalafil into 250ml volumetric flask. Added about 100ml methanol and mixed well for 30 minute to increase the solubility, Filtered and completed to the volume with methanol to get 0.4mg/ml, made further dilution. Then determination of tadalafil as described under HPLC method for determining tadalafil.

Procedure for industrial wastewater samples

To demonstrate the practical applicability of the proposed method, real industrial wastewater samples from the state company for pharmaceutical industries (NDI) Mosul-Iraq. were analyzed by spiked with the concentrations range 120-240 µg/ml of tadalafil and determination of tadalafil as described under HPLC method for determining tadalafil.

Results and discussion

The development of the HPLC method for the determination of drugs has received considerable attention in recent years because of its importance in routine quality control analysis. The aim of this study was to develop a rapid HPLC method for the determination of tadalafil in pure form ,its pharmaceutical formulations ,and environmental wastewater samples using the most commonly employed C_{18} column with UV detection. Different three analytical columns with various stationary phases were tested [C18 (octadecyl silane chemically bonded to porous silica,C8 (octyl silane chemically bonded to totally porous micro silica particles) and L11(phenyl groups chemically b0nded to porous silica particles)]. Good separation was achieved using a supelco RPC18 column. The latter was finally used for analysis. A HPLC method was proposed as a suitable method for the determination of tadalafil. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of methanol: water: triethylamine (60:38:2 v/v/v) ,adjust pH to 4 by phosphoric acid , accomplished at 220 nm. The retention time was 3.6 min at a flow-rate of 1.3 ml /min and the injection volume was 20 µl. . The mobile phase was chosen after several trials with other solvent combinations. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. Figures 2 shows a typical chromatogram obtained from the analysis of a standard of tadalafil using the proposed method.

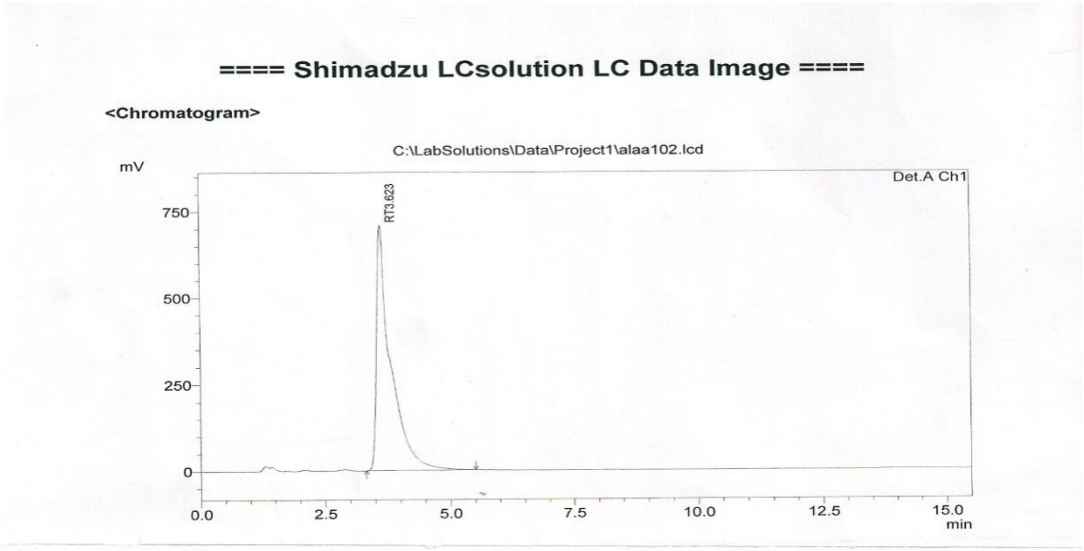


Figure 2. Typical chromatogram (tadafil 0.16mg/ml)

As shown in this figure, tadalafil was eluted forming symmetrical peak and well separated from the solvent front. Observed retention time (3.6min) allowed a rapid determination of the drug. System suitability parameters calculated under the optimized experimental conditions were capacity symmetry factor (tailing factor 1.05 and column efficiency (Theoretical plates) (n) =7650 plates/m. Table[1].

Linearity

To determine the linearity of the HPLC method, standard solutions of tadalafil were prepared. The linear ranges were found to be 0.04-0.28 mg /ml concentration, figure (3). The regression equation and correlation coefficient (r) obtained by least square regression method were y=966693x-285 (y: peak area, x: concentration) and 0.999, respectively. The regression equation calculated from calibration curves given with the standard deviations of slope (Sb) and intercept (Sa) on the ordinate are given in Table 1. The linearity of the calibration graph and conformity of HPLC value to Beer's Law were proven by the high correlation coefficient (r^2) for the regression equations indicating excellent linearity. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated[15] using the standard deviation of the intercepts(σ) and the mean slope (s) of the calibration curves. LOD=3.3σ/s and it was 0.04 µg/ml. and LOQ=10 σ/s and it was 0.12µg/ml.

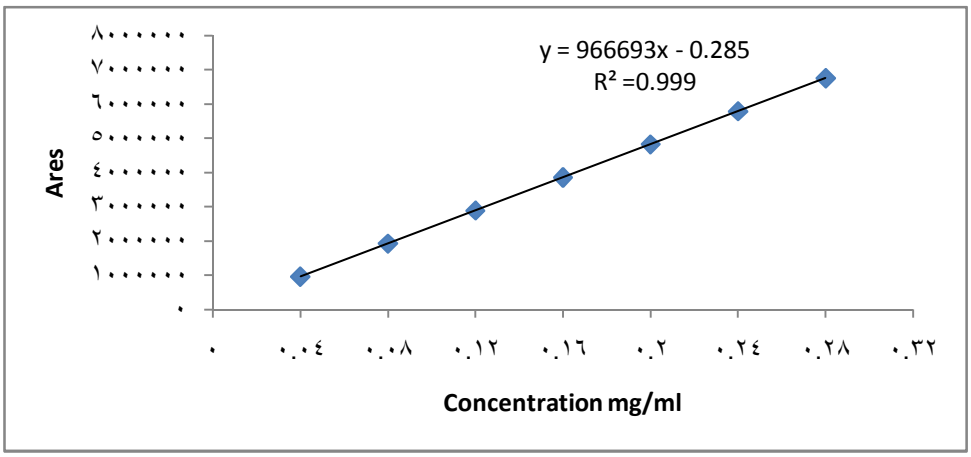


Figure 3. Calibration curve for tadalafil.

Table 1. HPLC conditions

Column	Supelco C_{18}(25cm×4.6mm),5 µm
Detector	220nm
Mobile phase	Methanol: Water: Tri ethylamine
Retention time	3.6min
Flow rate	1.3ml/min
Temperature	Ambient
Injection volume	20 µl
Tailing factor	1.05
Theoretical plate number (n)	7650 plates/m
Slope (sb)	966693
Intercept (sa)	-0.285
Correlation coefficient(r^2)	0.999
Beer s law limit mg/ml	0.04-0.28
RSD%	0.58
Accuracy(%recovery)	100±1.25
LOD µg/ml	0.04
LOQ µg/ml	0.12

Accuracy and precision

Repeatability is given as inter- and intra-day precision and accuracy evaluated by analyzing three different concentrations of tadalafil. Accuracy of the method was checked for six days at three concentration levels at 0.08, 016 and 0.24 mg/ ml in six replicates. The results are given in Table 2. The precision of the HPLC method was demonstrated by the relative standard derivation (RSD %) of lower than 1% for intra-day and inter-day. Recovery experiments were performed via standard-addition technique. To fixed and known amount of drug in the pre-analyzed tablet extracts, pure tadalafil (standard) was added at three levels and the total amount was found by the proposed method. The experiment at each level was repeated six times. The percent recoveries obtained are given in Table 3. Indicating the method is precise.

Table 2. Inter- and intra-day precision for tadalafil assay by the proposed HPLC method

Concentration of tadalafil mg/ml	Observed concentration of tadalafil *			
	Intra – day		Inter- day	
	Mean(n=6)	RSD%	Mean (n=6)	RSD%
0.08	0.079	0.48	0.081	0.15
0.16	0.163	0.75	0.161	0.52
0.24	0.244	0.91	0.242	0.95

*Mean of six determinations

Table 3. % Recovery of tadalafil assay by the proposed HPLC method

Amount added (mg)	Amount found(mg)*	% Recovery
0.08 0.16 0.24	0.081 0.158 0.242	101.25 98.75 100.83
Mean value		100±1.25

*Mean of six determinations

The proposed method was compared with other HPLC methods and found to be superior ,(Table 4).

Table 4. Comparison of the existing HPLC methods with the proposed method

Parameters	Method 1	Method 2	Method 3	Method 4
Ref	2	4	5	Proposed
Detector	260 nm	290 nm	280 nm	220 nm
Column	C18	C18	C18	C18
Linear range µg/ml	70-130	0.01-2	0.01- 8	40 -280
Mobile phase	Acetonitrile -Phosphate buffer (pH7) 60:40	Acetonitrile -Phosphate buffer (pH7) 35:65	Acetonitrile-Acetate buffer(pH2.6) 35:65	Methanol: Water: Tri-ethylamine(pH4) 60:38:2
Retention time	2.88 min	15 min	18 min	3.6 min
RSD%	< 2	< 10.7	———	< 1
Application	Tablet	Rat plasma	Human serum	Tablets and wastewater

Analytical application

The proposed method was successfully applied to the assay of tadalafil in tablets and wastewater samples. No interfering peaks were found in the chromatogram, indicating that the excipients did not interfere with the estimation of the drug by the proposed HPLC method. The results obtained are presented in table [5] , which reveals that there is close agreement between the results obtained by the proposed method and the label claim for the determination of tadalafil in pharmaceutical formulations and the results for wastewater samples ,table[6] show that good agreement between results and known values indicated the successful applicability of the proposed method for determination of tadalafil in environmental wastewater samples. And the recovery values obtained were closed to 100% .

Table 5. Determination of tadalafil in pharmaceutical formulations

Pharmaceutical formulations	Label amount (mg)	Found by proposed method *mg	Recovery%
Tablets [Tadananine(NDI)]	20mg/tab 10mg/tab	19.96 10.02	99.8 100.2

*Mean value of ten determinations

Table 6. Determination of tadalafil in wastewater samples

Wastewater samples	Added µg/ml	Found* µg/ml	Recovery %(n=10)
Industrial wastewater	80 160 240	80.8 159.2 243	101 99.5 101.25

* Mean value of ten determinations

Conclusion

This paper describes a reversed-phase HPLC method for determination of tadalafil in pure forms, pharmaceutical preparations and wastewater samples. The validation studies show good recoveries, precision and accuracy.. In summary, the reported method can be used for the routine quality control analysis of the investigated drug in pharmaceutical preparations and environmental wastewater samples.

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