Egypt. J. Chem. **Vol. 68**, No. 6 pp. 103 - 111 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Rapid HPLC Determination of Tadalafil, Avanafil, Sildenafil Citrate, And Vardenafil Hydrochloride Alone or in a Mixture

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Abstract

A simple, accurate, critical, and reliable RP-HPLC chromatographic method for the simultaneously determining tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride as a mixture of drug combinations or individually. Method separations were performed on an Agilent Zorbax L1 analytical column (4.6 mm × 150 mm, 5 microns) or equivalent. Using the filtration system and ultrasonic instrument a mixture of tetrahydrofuran: methanol: phosphate buffer pH 6.5 with triethylamine was used as the mobile phase. by using a room temperature of about 25 ℃, an adjusted flow rate of about 1.5 ml/min, and an ideal wavelength of 225 nm. In the chromatographic technique, the elution time of tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride was found to be 5.87, 9.08, 9.98, and 13.3 min, respectively, respectively, and the linearity range for tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride (25 – 75) µg/ml. The linear Regression coefficient for the method is ≥ 0.999. The validation method exercise complies with the parameters as per ICH guidelines: text and methodology Q2 (R1) and FDA guidelines. Hence, the proposed technique was used in quality control for all quantitative analyses of tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride which used for treatment erectile dysfunction (ED) in raw materials or other pharmaceutical dosage forms.

Keywords: Determination; Tadalafil, Avanafil, Sildenafil Citrate, Vardenafil hydrochloride; RAPID HPLC.

1. Introduction

One of the most common types of erectile dysfunction (ED) in men is age-related, and it affects more people as they become older. Therefore, the first line of treatment for erectile dysfunction (ED) caused by insufficient blood flow to the genitalia is phosphodiesterase type 5 inhibitors; PDE5 inhibitors are a class of drugs that are most commonly used to treat erectile dysfunction (ED). PDE5 inhibitors alter blood flow and cell communication throughout the body by blocking the PDE5 enzyme's ability to function; PDE5 inhibitors are medications that increase blood flow to specific bodily tissues, such as the penis. Despite being created initially to treat cardiovascular diseases including angina and high blood pressure, they are now the recommended course of treatment for men with erectile dysfunction (ED). The oral PDE5 (phosphodiesterase type 5) inhibitor family is a cornerstone of erectile dysfunction ED treatment. Vardenafil hydrochloride, avanafil, tadalafil, and sildenafil citrate are the four main PDE5 inhibitors.

Tadalafil, (6R-trans)-6-(1,3-Benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione as shown in (figure 1), [1]. Tadalafil is found as a white or almost white powder. Its molecular weight is 389.4, and its empirical formula is $C_{22}H_{19}N_3O_4$. Used to treat benign prostatic hypertrophy, pulmonary arterial hypertension, and erectile dysfunction (ED). The FDA initially authorized its usage in 2003 for erectile dysfunction, and again in 2009 for pulmonary arterial hypertension. Tadalafil has a longer half-life and more selectivity for PDE5 compared to other PDE5 inhibitors like sildenafil, making it a better alternative for chronic once-daily dosing in the treatment of pulmonary arterial hypertension. There are several methods for estimation of tadalafil by HPLC in pharmaceutical preparation, Tadalafil determined by RP- HPLC into bulk and pharmaceutical form samples [2-4]. In the presence of a degradation product, the estimate of tadalafil in bulk pharmaceutical dosage forms using HPTLC was successful. [5]. each study of toxicity and Photodegradation kinetics was done for tadalafil and sildenafil by simple HPLC method [6]. Tadalafil can be determined by UV-visible spectrophotometric by using dimethyl sulfoxide as solvent, absorbance at wavelength 285.6 nm [7]. This is only one of a few possible analytical methods for the analysis of tadalafil into many products found in above 50 commercial products plus wastewater [8-10].

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Received date 16 July 2023; revised date 02 March 2024; accepted date 30 October 2024
DOI: 10.21608/ejchem.2024.223119.8268

Avanafil,4-[(3-chloro-4-methoxy-benzyl)amino]-2-[(2S)-2-methylolpyrrolidin-1-yl]-N-(pyrimidine-2-ylmethyl)pyrimidine -5-carboxamide as shown in (figure 2), [11]. Avanafil is found as a white crystalline powder. Its molecular weight is 483.95, and its empirical formula is $C_{23}H_{26}ClN_7O_3$. The drug avanafil is used to treat erectile dysfunction. Nitric Oxide (NO), which is released during sexual stimulation, prompts a certain enzyme to create more cGMP, a chemical that has biological activity and dilates blood vessels. Erection results from this, and the corpus cavernosum fills with blood. However, PDE5 stops cGMP's effect by destroying it, which stops cGMP's action. In males with erectile dysfunction, avanafil inhibits PDE5, considerably extending the half-life of cGMP, and producing a strong erection. There are several techniques for estimation of avanafil by HPLC in drug preparation, successfully LC and LC-MS/MS Methods for quantitation determination of related compounds and avanafil into different dosage forms [11-13&18]. Increased oral bioavailability for avanafil by using excipients such as tween 80 improved dissolution profiles [14-15]. Formulation of nanoparticles of avanafil drug and also using stabilizers such as polyvinyl alcohol improved the solubility and invitro study [16]. An inexpensive approach for simultaneously separating avanafil and dapoxetine by HPLC instrument in pharmaceutical preparation and Biological Fluid [17].
Sildenafilcitrate,5-[2-Ethoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one citrate as shown in (figure 3), [19]. Sildenafil citrate is found in a white crystalline powder. Its empirical formula is $C_{28}H_{38}N_6O_{11}S$ and its molecular weight is 667. Sildenafil is used to treat erectile dysfunction by acting as a selective inhibitor of cyclic GMP-specific phosphodiesterase (Type V). There are several techniques for estimation of sildenafil by HPLC in drug preparation, HPTLC technique for determining sildenafil and its oxidized stress degradation product [20]. Other techniques to quantitative sildenafil and its related substance by using mobile phase consist of ethanol and water [21]. There are numerous ways to determining sildenafil and dapoxetine hydrochloride utilizing a mobile phase composed of buffer solution and a variable amount of acetonitrile. [22-24]. under stress conditions of tadalafil and sildenafil exposure, both products to ultraviolet radiation can be estimated as degradation products by simple liquid chromatography (LC) [25].

Vardenafil, 2- [2-ethoxy-5-(4-ethyl piperazine-4-ium-1-yl) sulfonylphenyl]-5-methyl-7-propyl-1H-imidazo [5,1-f] [1,2,4] triazin-4-one; chloride as shown in (figure 4), [26]. Vardenafil HCl was found as a white or slightly brown or yellow powder. Its empirical formula is $C_{23}H_{33}ClN_6O_4S$, and its molecular weight is 579.1. Vardenafil is used to treat erectile dysfunction by acting as a selective inhibitor of cyclic GMP-specific phosphodiesterase (Type V). There are several techniques for estimation of vardenafil by HPLC in drug preparation and urine in humans and rats, An accurate HPLC method by using a PDA detector at wavelength 300 nm can determined vardenafil in bulk form while mass spectrometry can determine vardenafil in urine [27-28]. Vardenafil may be detected in Human Plasma by validated LC/MS/MS [29]. To determine vardenafil and degradation product by using the Chromatographic method by gradient mobile phase in stress condition light, temperature, and hydrolysis [30]. This is only one of a few possible analytical methods for the analysis degradation product of vardenafil by using two pieces of equipment RP-UPLC and Mass spectrometry [31]. Combination formulations of dapoxetine and vardenafil were determined by two different methods HPLC and UV-Chemometric methods [32].

Aim of the study

This paper aims to create a simple and validated HPLC method to determine tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride found in most pharmaceutical dosage forms such as solutions, creams, ointments, injections, oral drops, and sprays.

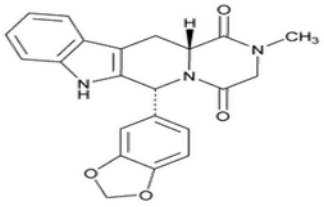

Figure 1: Structure of tadalafil

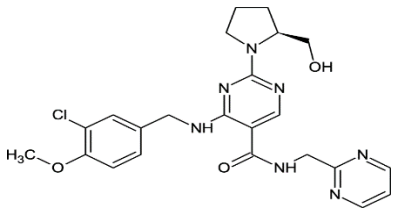

Figure 2: Structure of avanafil

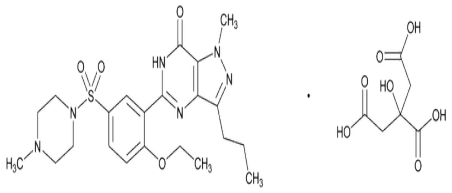

Figure 3: Structure of sildenafil citrate

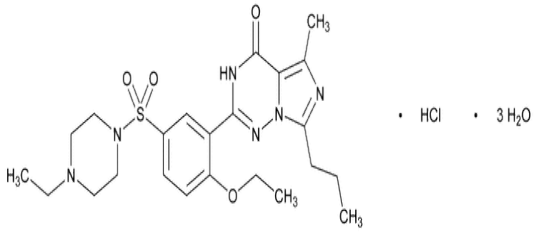

Figure 4: Structure of vardenafil

2. Materials and Methods

Reagents and chemicals

All materials and chemicals must be pharmaceutical grade. Purified water was used for the preparation of all solutions of the method. A pure sample of tadalafil, avanafil, sildenafil citrate, and vardenafil HCL was received from (YIHAN Industries Co-Ltd, China _ MSN Lab & PHALANX, and Ultra tech _ India). Methanol, tetrahydrofuran, and potassium dihydrogen orthophosphate (Fisher, UK).

Instruments

All the peak area measurements were made using Thermo scientific high-performance liquid chromatography vanquish (German), UV-visible spectrophotometer Shimadzu model 1650(Japan), Analytical balance model sartorius QUINTIX124-1SJP (Germany). The adjustment for mobile phase and buffer solutions using a pH instrument (Model pH 210 Hanna). The mobile phase mixture of (50:510:440V/V) tetrahydrofuran: methanol: phosphate buffer pH 6.5 with triethylamine was applied in isocratic mode, with a 0.45-µm nylon filter (Millipore) being used to filter the mobile phase, put into ultra-sonic for 20 min before use. The apply flow rate was about 1.5 ml/min and the injection volume was 20µL, the detector UV was adjusted at 225 nm and the method was achieved at Agilent zorbax L1 analytical, (4.6-mm × 150-mm; 5-Micron) and room temperature.

Standard solutions preparation

Dissolve about 5 mg of each tadalafil, avanafil, sildenafil citrate, and vardenafil HCL working standard in 5 ml mobile phase and complete to 20 ml measuring flask using mobile phase, to get final concentration and stirring for 5 min, then filter the solution by 0.20 µm nylon syringe filter (Millipore). Then, transfer 4 mL to a 20 ml measuring flask, and diluted with mobile phase to get the final concentration. {tadalafil, avanafil, sildenafil citrate, and vardenafil HCL (Conc 50:50:50:50 µg/ml)}

Analysis of synthetic formulation product (As Sample Solutions)

The synthetic formulation product contains 50 mg of tadalafil, 50 mg of avanafil, 50 mg of sildenafil citrate, and 50 mg of vardenafil hydrochloride. Take weight equivalent to 50 mg of each drug then put it into a 100 ml measuring flask. Complete the volume with the mobile phase then put the flask into ultra-sonic for 5 minutes. By using a filtration system filter the solution through a 0.20µm nylon syringe filter (Millipore). From the previous flask transfer 2 ml into a 20 ml flask and complete with the mobile phase to get a final concentration of 50µg/ml of each tadalafil, avanafil, sildenafil citrate, and vardenafil HCL. After enough time of saturation of the mobile phase into the column with the stated chromatographic conditions as described above. The sequence of injection was stopped after the complete separation of three areas, Peak areas were recorded. The analysis procedure was repeated three times with a synthetic formulation product.

3. Results and Discussion

Optimized chromatographic conditions

The HPLC method was precise for the simultaneously determining tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride. Both components give a good resolution factor with mobile phase tetrahydrofuran: methanol: phosphate buffer pH 6.5 with triethylamine at a ratio (50: 510: 440) v/v. The flow rate applied to the HPLC pump was 1.5 mL/minute and using wavelength at 225 nm. At accurate this wavelength, tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride can is quantified. Hence, 225 nm determined empirically is optimum. The average retention times for tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride were found to be 5.87, 9.08, 9.98, and 13.3 minutes, respectively, and mixture (Figures 5&6&7&8&9).

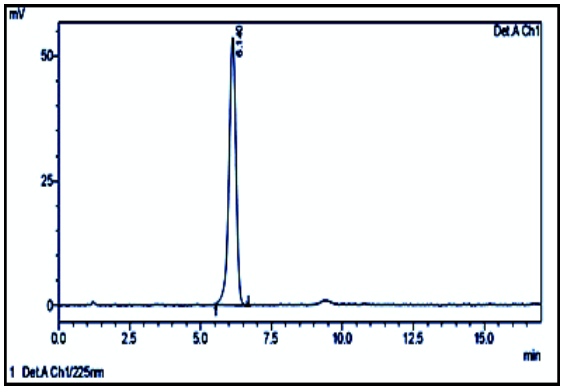

Fig. 5: Chromatogram of tadalafil

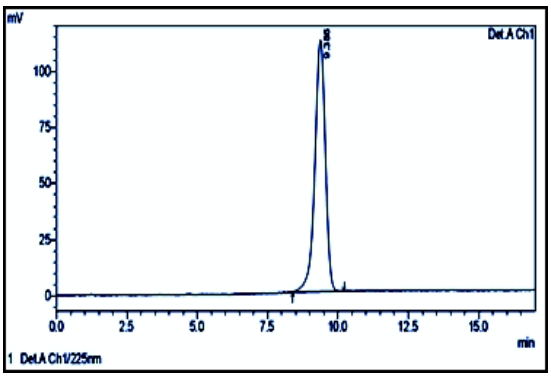

Fig. 6: Chromatogram of avanafil

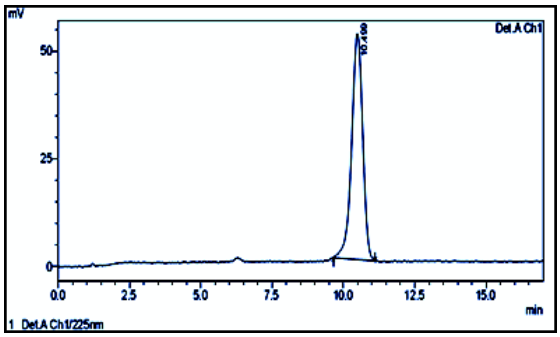

Fig. 7: Chromatogram of sildenafil citrate

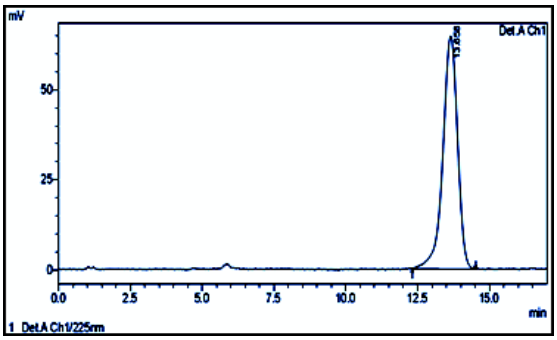

Fig. 8: Chromatogram of vardenafil HCl

Method Validation

Analytical Parameters

Table (1): Analytical parameters for tadalafil, avanafil, sildenafil citrate, and vardenafil hydrochloride

parameter	Tadalafil	Avanafil	Sildenafil citrate	Vardenafil HCl
System suitability				
Retention time (min)	6.007	9.063	9.938	12.937
Theoretical plate (N)	3245.667	3507.253	4000.263	3714.466
Tailing factor	1.0578	1.0332	1.0984	1.0958
Resolution factor	0	5.909	1.4108	4.0586
Capacity factor	0	0.5088	0.6544	1.1536
Linearity & regression data [33]				
Linearity range (µg/ml)	25-75	25-75	25-75	25-75
Slope(b)	1095.587774	3021.652877	1624.713042	2206.858349
Intercept(a)	-0.048755566	0.857708443	-0.762486769	-0.189402877
Correlation coefficient(R^2)	0.9999297	0.9998733	0.9995893	0.9999537
LOD	0.000822318	0.001103864	0.001988169	0.000667109
LOQ	0.002491874	0.003345041	0.006024754	0.002021542

Specificity

Tadalafil, avanafil, sildenafil citrate, and vardenafil HCl samples as well as a placebo of a chemical mixture were tested for method specificity. No interference is visible for the peaks of tadalafil, avanafil, sildenafil citrate, vardenafil HCl, or placebo in spectra analysis. For method specificity, the data obtained for tadalafil, avanafil, sildenafil citrate, and vardenafil HCl will therefore be deemed acceptable. As can be seen from the respective chromatograms (Figure 9-14)

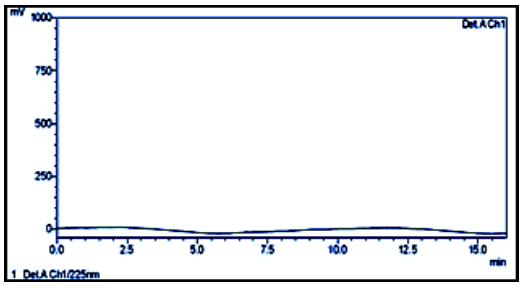

Fig. 9: Placebo without four drugs

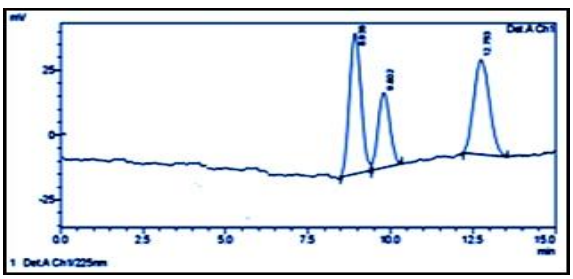

Fig. 10: Placebo without tadalafil

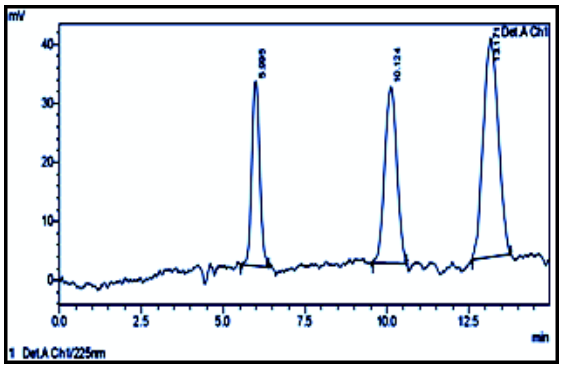

Fig. 11: Placebo without avanafil

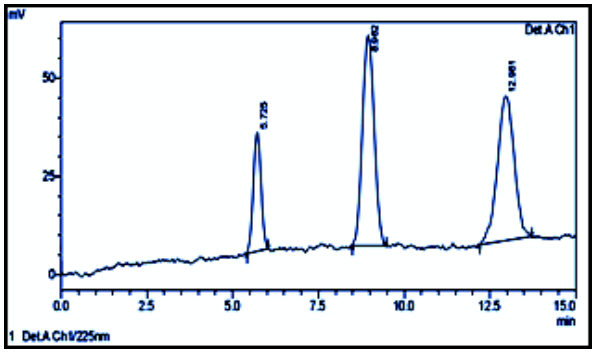

Fig. 12: Placebo without sildenafil

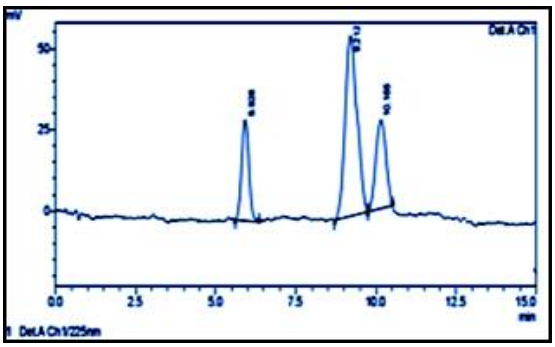

Fig. 13: Placebo without vardenafil

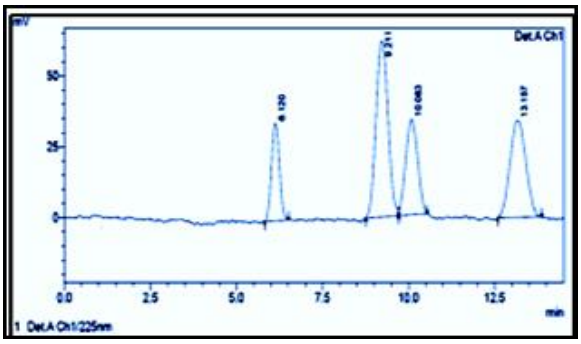

Fig. 14: Chromatogram of mixture STD

Effect of interference

The use of interference materials such as carbomer, glycerol, saccharin sodium, lactose, microcrystalline cellulose, magnesium stearate, crospovidone, titanium dioxide, talc, sodium starch glycolate, colloidal anhydrous silica, macrogol by adding 10 times excess with 5 mg/ml concentration of each drug show that there is no interference with each one.

Linearity and Range

Linearity for solutions is prepared using different concentrations of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl from (25-75 µml) in the range of 50 % to 150% of the theoretical quantity of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl solutions. A correlation coefficient should be N.L.T 0.999 of corresponding concentration versus the area response. From calibration curves coefficient of determination is equal to 0.999 so the method is linear which is shown in Figure 15, for tadalafil, figure 16 for avanafil, figure 17 for sildenafil, and Figure 18 for vardenafil HCl. This linearity test demonstrates a novel strategy for improved validations in accordance with Analytical method Validation ICH Q2 (R1) [33].

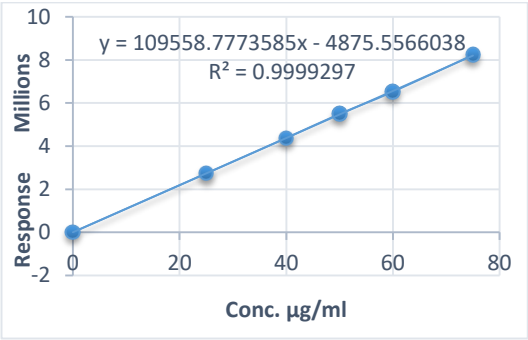

Fig. 15: Linearity curve for tadalafil

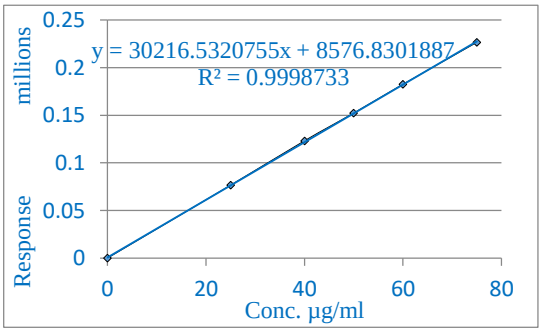

Fig. 16: Linearity curve for avanafil

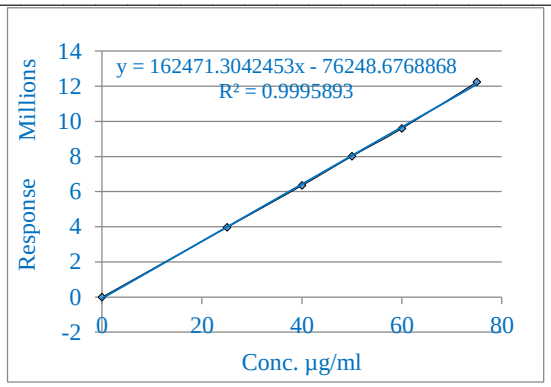

Fig. 17: Linearity curve for sildenafil citrate

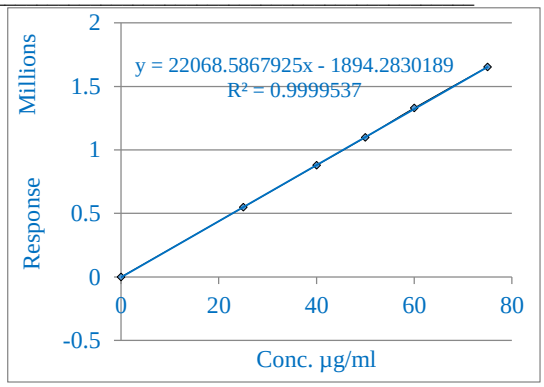

Fig.18: Linearity curve for vardenafil HCl

Accuracy and Recovery Studies

Samples are spiked by adding known quantities of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl standard to the placebo matrix containing all excipients of the product. The measurements are taken at three different concentrations 100%, 50 %, and 150 % in triplicate. The method's accuracy is determined by the percentage recovery of each concentration in comparison to the true values, the result is shown in Table (2)

Table (2): Accuracy and recovery result

Drugs	Weight. taken(µg)	Weight. found(µg)	% Recovery(n=3)	SD	% RSD
Tadalafil	25	25.89	99.71	1.4459	1.450
	50	51.14	99.23	0.0671	0.0676
	75	76.81	100.1	0.1605	0.1604
Avanafil	25	23.7	98.76	0.08178	0.083
	50	47.89	99.75	0.40114	0.401
	75	69.44	99.24	0.87023	0.870
Sildenafil citrate	25	23.05	100.21	0.5332	0.532
	50	47.26	100.56	0.6145	0.611
	75	74.92	99.89	0.6298	0.630
Vardenafil HCl	25	23.59	99.12	0.9931	0.403
	50	48.06	100.12	0.5057	0.505
	75	72.73	101.01	0.5431	0.537

Repeatability

Six replicates of the same solution at the same concentration were used to determine system precision for tadalafil, avanafil, sildenafil citrate, and vardenafil HCl (50 µg/ml). %RSD not more than 2%, the result is shown in Table 3.

Six preparations from a homogenous sample of the same concentration (50 µg/ml) of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl were used to determine the method precision. %RSD not more than 2 %, the result is shown in Table 4.

Table (3): System Precision Result

Name	Mean	SD	RSD
Tadalafil	560823.2	1641.6	0.2927
Avanafil	1478689.8	11132.2	0.7528
Sildenafil	772625.8	6025.05	0.7798
Vardenafil	1159220	7601.04	0.6557

Table (4): Method Precision Result

Name	Mean	SD	RSD
Tadalafil	491725.8	0.2911	0.2928
Avanafil	1359507.8	1.2864	1.2734
Sildenafil	805982.4	0.7804	0.7973
Vardenafil	1002059	0.6283	0.6234

Ruggedness:

Was performed by six replicates of a single sample of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl are implemented on the first day (Table 5), and then on a second day (Table 6), the method is rugged as the %RSD is not more than 2%.

Table (5): Ruggedness days 1

Name	Mean	SD	RSD
Tadalafil	571080.4	0.8462	0.8490
Avanafil	1566523.4	1.8013	1.7979
Sildenafil	796286.2	0.5835	0.5941
Vardenafil	1171262	0.1023	0.1016

Table (6): Ruggedness Day 2

Name	Mean	SD	RSD
Tadalafil	575877	0.242	0.2495
Avanafil	1558864.8	1.5865	1.5492
Sildenafil	802254.8	0.9255	0.9496
Vardenafil	1182117	0.33408	0.3265

Robustness

Performed by six replicates of a single sample of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl are implemented with a change in the column; the same analyst performs both tests. The method is rugged as the %RSD is not more than 3%, the result is shown in Tables 7&8.

Table (7): Robustness Column 1

Items	Mean	SD	RSD
Tadalafil	577342.6	0.1648	0.1634
Avanafil	1523106	0.8863	0.8799
Sildenafil	785416	0.5304	0.5458
Vardenafil	1139539	0.11712	0.1162

Table (8): Robustness Column 2

Items	Mean	SD	RSD
Tadalafil	568148.8	0.531	0.5340
Avanafil	1507152	1.1146	1.1145
Sildenafil	774629	0.3565	0.3609
Vardenafil	1126486	1.332	1.3128

Applications for drug

The proposed procedures which are sensitive and accurate can be determined by the studied substances in their synthetic pharmaceutical formulations and blood samples.

Table (9): determination of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl in pharmaceutical industrial samples

Drug Formulations	Applications							
	pharmaceutical formulations				blood samples			
	Taken (mg/ml)	Found (mg/ml)	Recom Method	% Recovery	Taken (mg/ml)	Found (mg/ml)	Recom Method	% Recovery
Tadalafil,(Cialis Tablets, 20mg)[a]	5	4.97	4.99	99.88	5	5.07	5.05	100.41
Avanafil(Erectawest Tablets, 100 mg)[b]	5	5.11	5.03	99.25	5	5.04	4.99	99.88
Sildenafil citrate (Westavarone Tablets, 100 mg)[b]	5	4.89	5.01	100.25	5	4.98	5.14	101.35
Vardenafil(Wetrecta ODT, 10 mg)[b]	5	5.23	5.12	100.17	5	5.18	5.11	100.67

Recommend method referring to pharmacopeia (BP 2022)

[a] Eli Lilly and Company, USA

[b] Western Egypt for trade and pharmaceutical industrial

4. Conclusion

Stability indicating RP-HPLC method is simple, provident, sensitive, and accurate has been advanced and validated for the simultaneous estimated of tadalafil, avanafil, sildenafil citrate, and vardenafil HCl individually or into a chemical mixture or separated pharmaceutical formulation and clustered we can use method for routine work of analysis into research centers and pharmaceutical factories for determined the four drugs so we can rename the method to become the general method for determined three drugs without any interference.

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