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Determinaton of sildenafil citrate and related substances in the commercial products and tablet dosage form using HPLC

N. Daraghmeh [a], M. Al-Omari [a], A.A. Badwan [a], A.M.Y. Jaber [b,*]

[a] *The Jordanian Pharmaceutical Manufacturing and Medical Equipment Co. Ltd, P.O. Box 94, Naor 11710, Jordan*
[b] *King Fahd University of Petroleum and Minerals, Chemistry Department, Dhahran 31261, Saudi Arabia*

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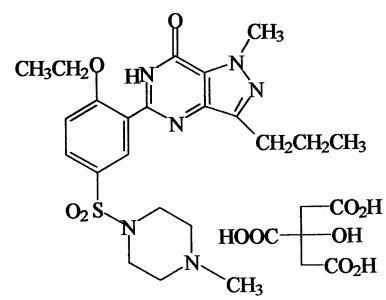
Abstract

This study aimed at developing and validating an HPLC method for the assay of sildenafil citrate and its related substances that might coexist in the drug commercial products and in tablets' formulation as impurities that originate from synthesis processes or degradation. A chromatographic system comprising a μBondapak C_{18} (10 μm) column, a mobile phase of ammonium acetate (pH 7.0, 0.2 M)–acetonitrile (1:1, v/v), a flow rate of 1 ml/min and a UV detector set at 240 nm has shown good chromatographic separation for sildenfil and the other related substances. The degree of linearity of the calibration curves, the percent recoveries of sildenafil and related substances, the limit of detection, LOD, and limit of quantitation, LOQ for the HPLC method have been determined. The HPLC method under study was found to be specific, precise, accurate, reproducible indicating stability and robust.

Keywords: Sildenafil; Viagra; HPLC; Tablet formulation

1. Introduction

Sildenafil citrate is a therapy used for erectile dysfunction by acting as a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). Sildenafil is chemically known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1 H-pyrazolo[4,3-d]pyrimidine-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate and has the following structural formula [1]



Sildenafil citrate

* Corresponding author. Tel.: +966-3-8602611; fax: +966-3-8604277.
E-mail address: amjaber@kfupm.edu.sa (A.M.Y. Jaber).

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As a preliminary investigation in this study, UV spectrophotometry and acid-base titration in aqueous and non-aqueous media proved to be successful techniques for the assay of sildenafil citrate. Sildenafil citrate dissolved in a mixture of methanol-water (30:70, v/v) showed maximum absorbance at about 290 nm. The drug-matrix (0.02 mg/l of a mixture of microcrystalline cellulose, dibasic calcium phosphate, magnesium stearate and croscarmelose sodium) showed a negligible response (about 1.1% of pure drug) at the same wavelength (290 nm) indicating the specificity of the spectrophotometric method towards sildenafil citrate. Standard solutions of sildenafil citrate only in the methanol–water solvent and solutions of sildenafil citrate and the drug-matrix in the same solvent have shown good calibration linearity over a range of 10–30 μg sildenafil citrate per ml with correlation coefficients of better than 0.9997. Furthermore, titration with 0.1 M NaOH and 0.1 M $HClO_4$ were found to be successful in determining the drug content of citric acid and sildenafil base, respectively. Although both the spectrophotmetric and the titration methods are appropriate for the determination of sildenafil citrate, they are not selective and the related compounds can not be differentiated from sildenadfil. Thus, a technique capable of separating the intact molecular from its related impurities is desired to establish a stability indicating method in bulk and dosage form. Reversed phase chromatography is the most commonly used for such separation in pharmaceutical preparations.

HPLC was used for the determination of sildenafil and its metabolite in plasma [2,3]. A reverse phase C_4 column of 100×4.6 mm (5 μm) in conjunction of a mobile phase made of a mixture of acetonitrile-water-0.5 M potassium phosphate buffer (pH 4.5) containing 0.01 M diethylamine hydrochloride (28:68:4, v/v/v) was used. The flow rate was 1.5 ml/min and the detector was set at a wavelength of 230 nm. The method was found to be selective, precise and linear over a range of 1–250 ng/ml. Preliminary trials of this study were carried out to use this method in the analysis of the potential impurities of sildenafil. The results indicated that most of the impurities have short retention time close to solvent front and they showed bad resolution from each other, thus the method reported is not stability indicating. A reversed-phase HPLC method was reported to be stability indicating for the determination of sildenafil citrate in the presence of its oxidative-induced degradation products [4]. This method was based on a C18 column (LiChrospher, 5 μm, 25 cm × 4.6 mm) and a mobile phase of 0.07 M potassium phosphate containing 0.1 M triehylamine (pH 3.0)-acetonitrile (70:30, v/v) and detection at a wavelength of 225 nm.

This work describes the development of a reversed-phase HPLC method that is suitable as a stability indicating for the determination of sildenafil citrate and its impurities that were originated from synthesis or degradation in either the drug commercial products or in the tablet dosage form.

2. Experimental

2.1. Instrumentation

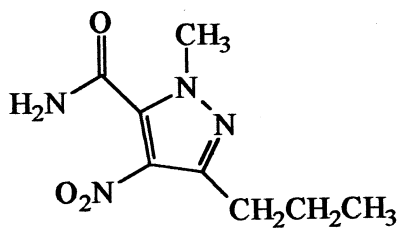
An HPLC unit equipped with a diode array 168 detector modules and programmable pump module 125 (System Gold, Beckman, USA) was used. The column utilised was Waters stainless steel (3.9×300 mm) packed with 10-μm packing L1 (μ Bondapak C18).

2.2. Materials

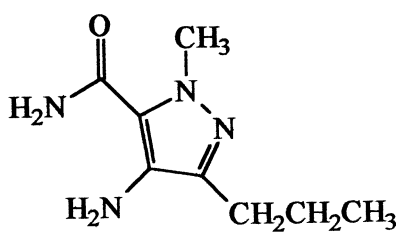
All the chemicals used were of pharmaceutical grade. Acetonitrile of chromnatographic grade and extra pure ammonium acetate were from Merck and ACROS, Belguim. Sildenafil citrate was obtained from JPM-Jordan. The inactive ingredients used as the drug-matrix include, microcrystalline cellulose type PH 200 of NF grade (FMC, Ireland), dibasic calcium phosphate of USP grade (Budenham, Germany), croscarmelose sodium of NF grade (FMC, Ireland) and magnesium stearate of NF grade (Malinkcrodt, USA), Opadry II blue (Colorcon, UK) used as a film material was composed of hydroxypropyl methylcellulose 2910, Lactose monohydrate, titanium dioxide, polyethylene glycol 3000, triacetin

FD&C blue No. 2 lake, quinoline yellow and carmolsine lakes.Viagra® tablets were obtained from the Jordanian market.

The compounds related to sildenafil which could be expected as impurities or might appear as degradation products have been prepared and identified at JPM. These compounds include, 1-methyl-4-nitro-3-*n*-propyl-5-pyrazole carboxamide (Impurity A); 4-amino-1-methyl-3-*n*-propyl-5-pyrazole carboxamide (Impurity B); 4-(2-ethoxy-benzoylamino) - 1 - methyl - 3 - *n* - propyl - 5 - pyrazole carboxamide (Impurity C); 5-(2-ethoxyphenyl)-1-methyl-3-*n*-propyl-pyrazole [4,3-d] pyrimidine-7-1 (Impurity D). The structural formulas of impurities, A, B, C and D are shown below.



Impurity A



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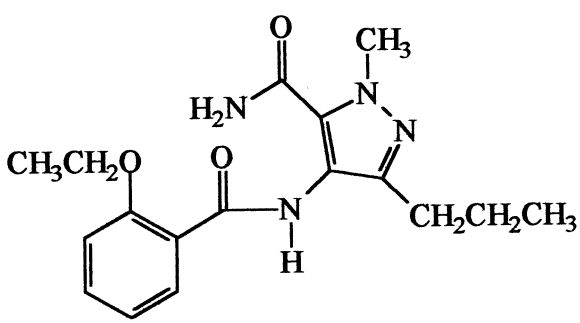
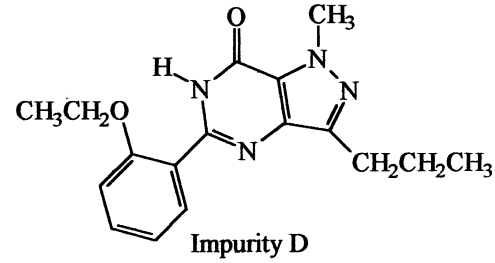


Impurity C



Impurity D

2.3. Optimised chromatographic conditions

Isocratic elution technique was utilised with the column that was maintained at room temperature. The mobile phase used was a mixture of ammonium acetate (pH 7.0, 0.2 M)-acetonitrile (1:1, v/v). The mobile phase was filtered and degassed by sonication before use. The flow rate was 1 ml/min. Samples of 20 μl were injected into the column and the detector was set at 240 nm. The relative standard deviation (R.S.D.) of six replicate injections of the standard preparation was not greater than 2.0% and the tailing factor was less than 3.0.

2.4. Preparation of solutions

2.4.1. Standard solutions of sildenafil citrate and related substances

An accurately weighed quantity of sildenafil citrate or related substances (Impurities A, B, C and D) was dissolved in the mobile phase and diluted quantitatively. Serial dilutions were carried out, using the mobile phase, to obtain solutions of known concentrations to be used for the standard preparation and the assay purposes.

2.4.2. Standard solutions of sildenafil citrate and related substances in the drug-matrix

Samples of about 100 mg sildenafil citrate or related substances were accurately weighed and mixed with appropriate proportions (1:2.5, w/w) of the drug-matrix components mentioned above. The mixtures were dissolved and diluted quantitatively to 100 ml using the mobile phase. The solutions were sonicated for 15 min, centrifuged at 4000 rpm for 10 min and the supernatant was used to prepare solutions of various quantities of sildenafil citrate using the mobile phase as a diluent.

2.4.3. Standard solutions of Viagra

Fifty tablets were powdered and accurately weighed portions equivalent to 100 mg sildenafil citrate were transferred to100 ml volumetric flasks. Mobile phase was added and the solutions were sonicated and centrifuged as above and the supernatant was used to prepare solutions of about 0.1 mg sildenafil citrate per ml using the mobile phase as the diluent.

2.5. Quantification

Equal volumes, (20 μl), of the standard preparations and the assay preparations that contain sildenafil citrate or related substances in the mobile phase were injected into the chromatograph and the chromatograms were recorded. The responses (peak area) for the major peaks were measured and the quantity of sildenafil or related substance in the assay solution was calculated from the equation $C_s(A_u/A_s)$ where A_u and A_s are the areas under the corresponding peaks and C_s is the concentration of sildenafil or related substance in the standard solution.

2.6. Linearity, limit of detection and limit of quantitation

Calibration graphs were constructed for sildenafil citrate and related substances in either standard solutions or synthetic mixtures of the drug product components. The degree of linearity was assessed by the correlation coefficient, *y*-intercept, slope, the confidence intervals for the slope and the intercept of the regression line [5,6].

The limit of detection, LOD and the limit of quantitation LOQ, have been estimated as 3 S.D. and 10 S.D. of the blank, respectively, according to the treatment by Miller and Miller [5].

3. Results and discussion

3.1. Developing the HPLC analytical method

The HPLC method carried out in this study aimed at developing a chromatographic system capable of eluting and resolving sildenafil and its impurities (related substances) from one another and that complies with the general requirements for system suitability.

The preliminary investigations were directed towards the effect of various variables on the system suitability of the method. The parameters assessed include the detection wavelength, the type and quantity of the organic modifier, the column, the salt concentration and the pH of the mobile phase.

Sildenafil citrate showed two UV-absorption maxima at 220–295 nm. The 240-nm wavelength showed a better resolution between the chromatographic peaks of sildenafil and its impurities and the absorption measurements at this wavelength were of optimal values for sildenafil citrate and other related compounds. The first trial was carried out by using reversed phase C_{18} column (Hypersil ODS, 5 μm, 250 × 4.6 mm) and a mixture of acetonitrile-0.1 M ammonium acetate (7:3, v/v). This system was found to be suitable to elute sildenafil but the retention time was short and the column resolution towards sildenafil and its impurities was low. As a means to increase the retention time, the percentage of acetonitrile portion in the mobile phase was decreased from 70 to 50%. The results showed that the retention time was increased but the tailing factor was so high for the sildenafil peak. However, the increase of the modifier ratio was associated by a decrease in the retention times of all the compounds. Replacing the acetonitrile organic modifier, with methanol was associated by a high tailing factor and longer retention times. When the Hypersil C_{18} column of 5 μm was then replaced by μBondapack C_{18} column of 10 μm, the tailing factor was reduced with better peak characteristics. Changing the concentration of ammonium acetate from 0.1 to 0.2 M had resulted in an enhancement to the peak symmetry and a reduction in the tailing factor. When the pH of the mobile phase was decreased from 7 to 5, the chromatographic peaks showed a decrease in the retention times and the column resolution for the impurities became low.

Consequently, the optimum chromatographic conditions mentioned previously were applied for all measurements. Fig. 1 (Chromatograms A and B) shows the significant separation of sildenafil and its related compounds using the optimised chromatographic system.

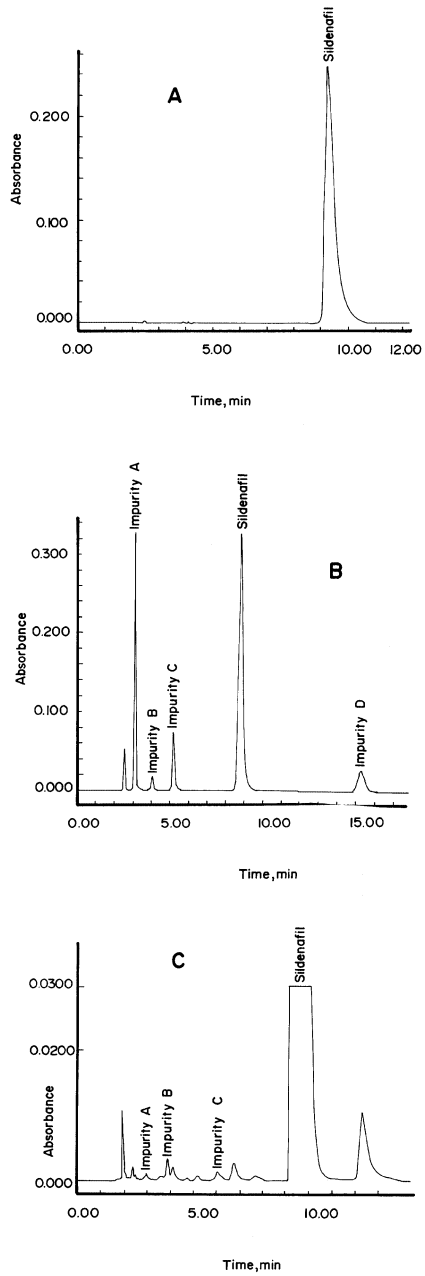


Fig. 1. HPLC chromatograms for sildenafil citrate in standard preparation (Chromatogram A), a mixture of sildenafil citrate and related compounds prepared in the drug-matrix solution (Chromatogram B) and for the raw sildenafil citrate dissolved in the drug-matrix solution (Chromatogram C).

The response factor (concentration/peak area), R_f, for each compound under study was determined at 240 nm using a concentration of about 5 μg/ml. The normalised R_f values (R_f for other compound/R_f for sildenafil), for sildenafil and impurities A, B, C and D were 1.0, 3.8, 1.6, 1.3 and 1.1.

3.2. Specificity

The HPLC chromatograms recorded for the drug-matrix (mixture of the drug-excipients) showed almost no peaks within a retention time range of 20 min. Fig. 1 shows a representative chromatogram for sildenafil alone (Chromatogram A) and a chromatogram (Chromatogram B) for a mixture of sildenafil with its related compounds, A, B, C and D (a mixture containing about 15 μg/ml of each component). The figure shows that sildenafil is clearly separated from its related compounds and these four compounds are also well separated from each other. Thus, the HPLC method presented in this study is selective for sildenafil citrate and the other four related compounds, which might coexist as impurities originated from the synthesis processes.

The specificity was also demonstrated by induced degradation of sildenafil citrate samples by treating them with either 1.5% H_2O_2 and storing the sample at room temperature for 60 min, 0.1 M HCl and storing at 65°C for 12 days, or 0.1 M NaOH, and storing at 65°C for 12 days or by heating a pure solid sildenafil citrate sample at the melting point for 5 min. The recovery of sildenafil was 101.7 and 99.3% with a total degradation of 0.2 and 0.4% in the case of 0.1 M HCl and 0.1 M NaOH, respectively, however the decomposition was significant when sildenafil was treated with 1.5% H_2O_2 or heated at the melting point where the sildenafil recovery was 65.0 and 90.0% and the total degradation was 35.0 and 13.0%, respectively. Thus, the degradation products will be resolved well from sildenafil.

The possible photodegradation of sildenafil citrate in the solid form and in solutions of the mobile phase (samples of 1.5 mg/ml) was studied by exposing various samples of sildenafil citrate to direct sunlight and dark for 6 days. Exposure of sildenafil citrate powder to light showed an almost-

Table 1
Linearity of calibration curves for sildenafil citrate and its related compounds in standard preparations and in the drug-matrix preparation. Number of points in the regression line is 5 for each case

Compound	Calibration range (μg/ml)	Correlation coefficient	Slope	95% confidence intervals of the slope[a]	Intercept	95% confidence intervals of the intercept[a]	LOD (μg/ml)	LOQ (μg/ml)
Standard preparations								
Sildenafil citrate	64.3–257.0	0.9999	0.679	±0.0116	−0.253	±2.001	2.31	7.70
Sildenafil citrate	2.39–11.95	0.9995	0.939	±0.0535	0.1991	±0.391	0.413	1.38
Impurity A	3.42–17.13	0.9999	0.263	±0.00369	0.0102	±0.0386	0.145	0.485
Impurity B	3.38–16.89	1.000	0.625	±0.00473	0.0498	±0.0488	0.0773	0.258
Impurity C	3.51–17.57	1.000	0.757	±0.00484	0.0939	±0.0520	0.0680	0.227
Impurity D	2.97–14.87	0.9999	0.898	±0.0268	0.1775	±0.244	0.268	0.895
Drug-matrix preparations								
Sildenafil citrate	64.3–257.0	0.9999	0.671	±0.0180	1.312	±3.122	3.65	12.15
Sildenafil citrate	2.39–11.95	0.9997	0.959	±0.0597	0.0142	±0.436	0.451	1.68
Impurity A	3.42–17.13	0.9999	0.265	±0.00444	0.0015	±0.0465	0. 173	0.578
Impurity B	3.38–16.89	0.9999	0.629	±0.00934	0.0294	±0.0965	0. 152	0.506
Impurity C	3.51–17.57	0.9999	0.763	±0.0112	0.0482	±0.121	0. 156	0.521
Impurity D	2.97–14.87	0.9999	0.916	±0.0158	0.0349	±0.144	0. 156	0.519

[a] Confidence intervals of the slope and the intercept = (S.D. of the slope or intercept × t); The value of t at 3 degrees of freedom and 95% confidence level is 3.18.

full recovery without a significant degradation. However, sildenafil citrate sample solutions showed a photodegradation of about 0.7% (two photodegradation products at relative retention times of 0.65 and 0.84) and an almost complete recovery. The samples kept in dark did not show a significant sign of degradation. Sildenafil citrate sample solutions kept in dark at 40°C did not show a significant degradation indicating that the degradation took place in the sunlight was solely due to the light effect.

Thus, the method developed here is stability indicating since it allows the separation of sildenafil from related compounds and degradation products and it allows the quantitative determination of sildenafil in the presence of, or separate from its impurities.

3.3. Linearity

The linearity of calibration curves (peak area vs. concentration) for sildenafil citrate in pure solutions as well as in the drug-matrix solutions were checked over the concentration ranges of about 64–257 and 2–12 μg/ml and found to be linear with correlation coefficients of better than 0.999 in most cases (Table 1). Furthermore, the linearity of the calibration curves for the compounds related to sildenafil were also studied over the ranges between 2 and 17 μg/ml and found to be linear with correlation coefficients of better than 0.9999 for all the cases in either pure solutions or in solutions comprising the drug-matrix (Table 1). Table 1 lists the linearity parameters of the calibration curves for sildenafil citrate and related compounds in pure and drug-matrix solutions.

3.4. Sensitivity and accuracy

The limits of detection LOD and limits of quantitation, LOQ, were calculated for the calibration graphs of sildenafil and its related compounds as three and ten times of the noise level for LOD and LOQ, respectively [5,6]. The values for LOD and LQD are given in Table 1.

The accuracy of the method was tested by analysing different samples of sildenafil citrate and all the other related compounds at various concentration levels in either pure solutions or in solutions comprising the drug-matrix used in tablet formulation. The results were expressed as percent recoveries of the particular components in the samples (Table 2). Table 2 shows that the overall percent recoveries of sildenafil in pure and drug-matrix solutions were 100.7 (relative standard deviation (R.S.D.) = 2.41%) and 99.9 (R.S.D. = 1.89%), respectively. However, the related compounds showed the overall percent recoveries ranging from 99.5 to 100.4 with R.S.D.s ranging from 0.61 to 2.21%.

3.5. Stability of analytical solutions

Sample solutions of sildenafil in pure and drug-matrix solutions were tested for HPLC stability over 24 h. The samples were analysed by the optimised HPLC method in fresh and stored solutions. The percent difference observed was in the range of -0.31 to -0.76 (Table 3), indicating the possibility of using standard solutions of sildenafil citrate in pure or drug-matrix solutions over a period of 24 h without degradation.

3.6. Repeatability and reproducibility

Various samples containing about 160 μg/ml sildenafil citrate in a synthetic matrix (drug-matrix) were analysed by three independent analysts (six samples each) over 1 day and various days. The 1 day repeatability gave the overall percent recoveries of 100.1, 101.2% and 99.9 with %R.S.D. of 1.2, 0.62 and 0.26, respectively. The long-term reproducibility for all the analysis gave an over all recovery and R.S.D. of 100.4% and 0.96%, respectively.

3.7. Robustness

The optimum HPLC conditions set for this method have been slightly modified for samples of sildenafil citrate (160 mg/ml) and related compounds (7–9 mg/ml) dissolved in the drug matrix as a means to evaluate the method ruggedness.

The small changes made included: the mobile phase ratio, the flow rate, the detection wave

Table 2
Validation of the HPLC method for the determination of sildenafil citrate and its related compounds in standard or drug-matrix solutions

Compound	Quantity added (μg/ml)	Standard solutions		Drug-matrix Solutions	
		Quantity found (μg/ml)	Recovery (%)	Quantity found (μg/ml)	Recovery (%)
Sildenafil citrate	2.39	2.52	105.4	2.43	101.7
	3.59	3.70	103.1	3.56	99.2
	5.98	5.98	100.0	5.74	96.0
	8.37	8.06	96.3	8.27	98.8
	11.95	11.81	98.8	11.79	98.7
	64.26	64.63	100.6	65.99	102.7
	128.52	129.60	100.8	129.13	100.5
	160.65	160.56	99.9	160.98	100.2
	192.78	194.73	101.0	195.55	101.4
	257.04	259.04	100.8	257.35	100.1
Overall recovery			100.7		99.9
%R,S.D.			2.41		1.89
Impurity A	3.43	3.38	98.5	3.49	101.7
	5.14	5.15	100.2	5.11	99.4
	8.56	8.56	100.0	8.50	99.3
	11.99	11.93	99.5	11.99	100.0
	17.13	16.97	99.1	17.15	100.1
Overall recovery			99.5		100.1
%R.S.D.			0.688		0.962
Impurity B	3.38	3.39	100.3	3.41	100.9
	5.07	5.11	100.8	5.13	101.2
	8.44	8.44	100.0	8.38	99.3
	11.82	11.76	99.5	11.83	100.1
	16.89	16.77	99.3	16.87	99.9
Overall recovery			99.98		100.3
%R.S.D.			0.606		0.769
Impurity C	3.51	3.56	101.4	3.55	101.1
	5.27	5.30	100.6	5.32	100.9
	8.78	8.78	100.0	8.68	98.9
	12.30	12.21	99.3	12.25	99.6
	17.57	17.37	98.9	17.47	99.4
Overall recovery			100.0		100.0
%R.S.D.			1.00		0.968
Impurity D	2.97	2.99	100.7	2.99	100.7
	4.46	4.64	104.0	4.49	100.7
	7.43	7.43	100.0	7.33	98.7
	10.41	10.26	98.6	10.39	99.8
	14.87	14.65	98.5	14.77	99.3
Overall recovery			100.4		99.8
%R.S.D.			2.214		0.876

length, the sonication time, the filtration system and the column (Table 4). Table 4 shows that the percent recoveries of sildenafil and related compounds were good under most conditions and did not show a significant change when the critical parameters were modified. The tailing factor for sildenafil and the related compounds was always less than 2.6 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature would conclude that the method conditions are robust.

Table 3
Stability of sildenafil citrate in standard and drug-matrix solutions over a period of 24 h[a]

Sample	Quantity added (μg/ml)	Quantity found (μg/ml)		Difference (%)
		Fresh solution	Stored solution	
Standard solutions of sildenafil citrate	64.26	64.63	64.83	−0.31
	160.7	160.6	160.8	−0.12
	257.0	259.0	258.6	0.15
Standard solutions of sildenafil in the drug-matrix	64.26	66.0	65.5	0.76
	160.7	161.0	160.3	0.43
	257.0	257.4	256.4	0.39

[a] Difference (%) = (quantity found in fresh solution − quantity found in stored solution)/(quantity found in fresh solution) × 100.

Table 4
Effect of experimental parameters on the percent recoveries of sildenafil citrate and its related compounds[a]

Parameter	Modification	Sildenafil % Recovery	Impurity A % Recovery	Impurity B % Recovery	Impurity C % Recovery	Impurity D % Recovery
Mobile phase ratio (v/v)	Buffer:ACN					
	60:40	100.4	103.1	97.9	96.7	98.6
	55:45	99.8	98.7	92.9	97.3	96.8
	50:50	100.4	98.3	99.9	95.2	95.0
	45:55	99.5	101.0	103.9	99.2	96.2
	40:60	99.3	98.4	104.5	100.2	92.5
PH	6.5	98.8	92.6	100.6	103.0	96.3
	7.0	100.4	100.5	102.0	101.9	105.4
	7.5	100.5	101.0	102.2	102.8	101.3
Flow rate (ml/min)	0.5	99.4	102.7	101.2	99.4	101.7
	1.0	100.4	100.5	102.0	101.9	105.4
	1.5	99.8	100.9	102.7	101.7	99.6
Wavelength (nm)	235	99.8	100.2	105.0	102.4	105.2
	240	100.1	100.5	102.0	101.9	105.4
	245	99.7	99.9	107.9	104.0	98.5
Soniction time (min)	10	100.3	98.9	99.7	99.3	102.4
	15	100.4	100.5	102.0	101.9	105.4
	20	102.4	100.1	100.8	108.8	104.3
Filtration system	Nylon	99.9	99.4	102.9	101.0	102.6
	Centrifuge	99.4	100.5	102.0	101.9	105.4
	PTFE	100.7	99.6	101.2	101.9	104.5
Column type	Used column	100.1	100.1	99.9	99.6	99.6
	New column	100.4	98.3	99.9	95.2	95.0

[a] ACN, acetonitrile; PTFE, polytetrrafluoroethylene.

3.8. Application

The validity of the method developed here for sildenafil and the related substances that might interfere in the determination of sildenafil was studied by assaying a commercial sildenafil citrate product and Viagra® tablets from the Jordanian market. Fig. 1 (Chromatogram C), shows an HPLC chromatogram for sildenafil citrate raw material obtained from one of the suppliers. Three of the compounds related to sildenafil appeared clearly on the chromatogram; this indicates that the proposed method can differentiate between the active moiety and its related impurities. Samples of Viagra® ($n = 6$) were analysed for sildenafil by this method and the results showed a percent recovery of 100.1 and a R.S.D. of 0.60%.

4. Conclusion

An HPLC method for the assay of sildenafil citrate and its related compounds in the commercial drug products and in the tablet formulation was validated in this study. Sildenafil citrate and the other related compounds which may coexist with it as impurities or as degradants gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability. Slight changes in the experimental conditions did not affect significantly the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method. All the statistical values (percent recovery, RSD, %D, confidence intervals of the slope and the intercept, LOD and LOQ) calculated were within the acceptable limits. The method can be used for the assay and chromatographic purity of the drug substance in its solid dosage form and for quality control purposes.

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